

CORPORATE SOURCE: Inst. Mol. Genet., Czech. Acad. Sci., Prague, Czech.
 SOURCE: Mol. Reprod. Dev. (1992), 33(2), 165-71
 CODEN: MREDEE; ISSN: 1040-452X

DOCUMENT TYPE: Journal
 LANGUAGE: English

AB Sperm coating proteins of 16, 17, and 19 kDa have been purified from boar seminal plasma. The 17 kDa **protein** has been identified as an antigen recognized by monoclonal antibody ACR.3 and is thus identical to low mol. mass **zona pellucida binding protein** from boar spermatozoa (Moos, J., et al., 1990). The 17 and 19 kDa **proteins** are **glycosylated** and tend to form hetero-complexes. The 17 kDa ACR.3 antigen is sequentially released from the sperm cell surface during capacitation and, after induction of the acrosome reaction, the 16 kDa form was also obsd. Immunocytochem. studies on boar reproductive tissues have suggested that the seminal vesicle epithelium may be the source of these proteins.

L8 ANSWER 8 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1988:434792 HCAPLUS
 DOCUMENT NUMBER: 109:34792
 TITLE: Monoclonal antibodies specific for an oviductal component associated with the hamster zona pellucida
 AUTHOR(S): St-Jacques, Sylvie; Bleau, Gilles
 CORPORATE SOURCE: Dep. Obstet. Gynecol., Univ. Montreal, Montreal, PQ, Can.
 SOURCE: J. Reprod. Immunol. (1988), 12(4), 247-61
 CODEN: JRMIDR; ISSN: 0165-0378

DOCUMENT TYPE: Journal
 LANGUAGE: English

AB Five monoclonal antibodies (MAbs) were produced against oviductal **zona pellucida** (ZP) of the hamster. They were purified from ascitic fluid by HPLC on hydroxylapatite and anion-exchange columns. All 5 MAbs reacted selectively with oviductal ZP and oviductal secretions; no binding was obsd. to intra-ovarian ZP. A study of the tissue specificity, as evaluated by indirect immunofluorescence, revealed the binding of all of these Abs only to the oviduct and, to a lesser extent, to the uterus. A cytosolic fraction from hamster oviduct was subjected to SDS-PAGE under reducing conditions and electro-transfer to a nitrocellulose membrane; immunoenzymic staining showed a reaction with a polydispersed oviductal component of high mol. wt. (.apprx.200,000). The native antigen has a mol. wt. >400,000 as detd. by mol. sieve chromatog. Thus, an oviductal antigen is added to the hamster ZP during its transit through the oviduct. This antigen, called oviductin, is a heavily **glycosylated protein** of high mol. wt.

L8 ANSWER 9 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1980:161203 HCAPLUS
 DOCUMENT NUMBER: 92:161203
 TITLE: Synthesis of zona pellucida proteins by denuded and follicle-enclosed mouse oocytes during culture in vitro
 AUTHOR(S): Bleil, Jeffrey D.; Wassarman, Paul M.
 CORPORATE SOURCE: Dep. Biol. Chem., Harvard Med. Sch., Boston, MA, 02115, USA
 SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1980), 77(2), 1029-33
 CODEN: PNASA6; ISSN: 0027-8424

searcher : m.smith 83278

DOCUMENT TYPE: Journal
LANGUAGE: English

AB To det. whether the zona pellucida originates from the oocyte, surrounding follicle cells, or both, denuded and follicle-enclosed mouse oocytes at various stages of growth were isolated and cultured in vitro in the presence of either methionine-35S or fucose-3H to det. the site of synthesis of the 3 recently identified zona pellucida proteins, ZP1, ZP2, and ZP3. Approx. 1.5% of the methionine-35S, and as much as 45% of the fucose-3H, that was incorporated into TCA-insol. material by denuded or follicle-enclosed oocytes during a 12-h culture period was assocd. with zonae pellucidae removed from the cultured oocytes. Incorporation of methionine-35S into zona pellucida proteins was depressed to <1/50th when denuded oocytes were cultured in the presence of puromycin, and secretion of zona pellucida proteins by denuded oocytes was demonstrated by pulse-chase expts. Na dodecyl sulfate-polyacrylamide gel electrophoresis of methionine-35S- and fucose-3H-labeled proteins present in oocytes, zonae pellucidae, and follicle cells revealed that denuded oocytes synthesize and secrete zona pellucida proteins, whereas no evidence was obtained to suggest that follicle cells synthesize these proteins. Denuded oocytes, ranging in diam. from 48 to 68 .mu., incorporated both methionine-35S and fucose-3H into zona pellucida proteins during culture in vitro, whereas zonae pellucidae removed from fully-grown oocytes (85 .mu.) were not radiolabeled to a significant extent. After culture of denuded or follicle-enclosed oocytes for 12 h, >95% of the fucose-3H incorporated into oocyte proteins was found in ZP1, ZP2, and ZP3, indicating that zona pellucida proteins are the major class of **proteins glycosylated** during oocyte growth. Apparently, the zona pellucida originates from the mammalian oocyte itself, rather than from the surrounding follicle cells.

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L3      1 SEA FILE=REGISTRY "GLYCOSYLATED PROTEINS"/CN
L4      185 SEA FILE=HCAPLUS L1 OR L2 OR (HUMAN(L) (ZONA(L) PELLUCIDA(L)
        PROTEIN OR ZP3)) OR RHZP?
L5      2170 SEA FILE=HCAPLUS GLYCOSYLATED(2A) (PROTEIN? OR PEPTIDE?) OR L3
L6      2 SEA FILE=HCAPLUS L4 AND L5
L7      746 SEA FILE=HCAPLUS (ZONA(2A) PELLUCIDA(L) PROTEIN OR ZP3) OR
        RHZP?
L8      9 SEA FILE=HCAPLUS (L5 AND L7) NOT L6
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=> d ibib abs hitrn l6 1-2

L6 ANSWER 1 OF 2 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:226653 HCAPLUS
DOCUMENT NUMBER: 131:98261
TITLE: Cloning and characterization of a zona pellucida 3
cDNA from a marsupial, the brushtail possum
Trichosurus vulpecula
AUTHOR(S): McCartney, Carmen A.; Mate, Karen E.
CORPORATE SOURCE: Cooperative Research Centre for Conservation and
Management of Marsupials, School of Biological
Sciences, Macquarie University, 2109, Australia

searcher : m.smith 83278

SOURCE: Zygote (1999), 7(1), 1-9
 CODEN: ZYGOEB; ISSN: 0967-1994
 PUBLISHER: Cambridge University Press
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB The mammalian **zona pellucida** (ZP) is an extracellular glycoprotein coat that plays vital roles throughout fertilization and preimplantation development. Like that of eutherian mammals the brushtail possum ZP is composed of three **glycosylated proteins** of 137 kDa, 92 kDa and 62 kDa. The 62 kDa **protein** is a **ZP3** ortholog based on its nucleotide and deduced amino acid sequence. The brushtail possum **ZP3** cDNA isolated in this study is 1305 nucleotides with an open reading frame encoding a 422 amino acid peptide of 45.7 kDa. Possum **ZP3** has a 46% amino acid identity with eutherian **ZP3** and shares similar structural characteristics including 12 conserved cysteine residues, N-linked glycosylation sites and hydrophobic regions. Like **human** and rabbit ZP1 an altered furin cleavage site upstream of the C-terminal hydrophobic domain also occurs in possum **ZP3** (S-R-K-R), suggestive of processing by a furin-related endoprotease. Expression of brushtail possum **ZP3** is limited to the ovary. Characterization of brushtail possum **ZP3** will enable examn. of its functional role in marsupial fertilization and its effectiveness as an immunocontraceptive agent.

L6 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2000 ACS
 ACCESSION NUMBER: 1998:404617 HCAPLUS
 DOCUMENT NUMBER: 129:187090
 TITLE: Modeling human sperm-egg interactions in vitro: signal transduction pathways regulating the acrosome reaction
 AUTHOR(S): Benoff, Susan
 CORPORATE SOURCE: Department of Obstetrics and Gynecology, North Shore University Hospital-New York University Medical College, Manhasset, NY, 11030, USA
 SOURCE: Mol. Hum. Reprod. (1998), 4(5), 453-471
 CODEN: MHREFD; ISSN: 1360-9947
 PUBLISHER: Oxford University Press
 DOCUMENT TYPE: Journal; General Review
 LANGUAGE: English

AB A review with many refs. Recent advances in characterizing sperm surface receptors and ion channels, when combined with the rapidly expanding knowledge of interactions among second messenger systems in somatic cells, permit formulation of a tentative mol. mechanism for the regulation of the **human** sperm acrosome reaction. As spermatozoa pass through the cumulus mass, progesterone binds to its sperm surface receptor, alkalinizes the sperm head cytosol and potentiates changes in intracellular ionized calcium. Primary binding of spermatozoa to egg involves receptors for mannosyl, N-acetylglucosaminyl and, possibly, fucosyl residues of the **glycosylated zona protein, ZP3**. These receptors aggregate on multivalent ligand binding, migrate to the equatorial region along an actin filament network formed between the plasma and acrosomal membranes during capacitation, and activate a G protein/protein kinase A/protein kinase C second messenger system and a secondary proteolysis signal. Binding of a receptor tyrosine kinase to **ZP3** amino acid residues simultaneous with the sugar recognition event triggers tyrosine phosphorylation signalling. All signals combine to open a voltage-dependent calcium channel. The

* date
 priority 2/19/98

resulting elevated calcium signal depolymerizes the inter-membrane actin network and activates phospholipases, leading to an acrosome reaction.

=> d ibib abs hitrn l8 1-9

L8 ANSWER 1 OF 9 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:608306 HCAPLUS
DOCUMENT NUMBER: 131:335874
TITLE: Expression of a Recombinant Porcine Zona Pellucida Glycoprotein ZP1 in Mammalian Cells
AUTHOR(S): Tsubamoto, Hiroshi; Yamasaki, Noriyuki; Hasegawa, Akiko; Koyama, Koji
CORPORATE SOURCE: Department of Obstetrics and Gynecology, Hyogo College of Medicine, Nishinomiya, 663-8501, Japan
SOURCE: Protein Expression Purif. (1999), 17(1), 8-15
CODEN: PEXPEJ; ISSN: 1046-5928
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Porcine zona pellucida glycoprotein (pZP1) is a good candidate for a contraceptive vaccine. For the purpose of producing glycosylated pZP1, several types of recombinant pZP1 proteins were produced in mammalian cell lines. In the first expt., a minigene encoding pZP1 (681 amino acids) was designed for insertion into an expression vector and then transfected to three cell lines (293T, CHO-K1, and LLC-PK1). The resulting recombinant **proteins** were highly **glycosylated** and were localized in the cytoplasm. To produce a secretory type of recombinant pZP1, in the second expt., a cDNA coding for pZP1 excluding a putative transmembrane region and a smaller cDNA coding for 1-198 amino acid residues of pZP1 were designed to produce fusion proteins with the human IgG1 heavy chain. The resultant recombinant proteins were secreted into the supernatant from both transfected cell cultures. Recombinant secretory proteins are useful because of their simple affinity purifn. (c) 1999 Academic Press.

L8 ANSWER 2 OF 9 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1996:205023 HCAPLUS
DOCUMENT NUMBER: 124:312986
TITLE: Evaluating **zona pellucida** structure and function using antibodies to rabbit 55 kDa ZP **protein** expressed in baculovirus expression system
AUTHOR(S): Prasad, Sarvamangala V.; Wilkins, Brendan; Skinner, Sheri M.; Dunbar, Bonnie S.
CORPORATE SOURCE: Dep. Cell Biol., Baylor Coll. Med., Houston, TX, 77030, USA
SOURCE: Mol. Reprod. Dev. (1996), 43(4), 519-29
CODEN: MREDEE; ISSN: 1040-452X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A cDNA encoding the rabbit 55 kDa ZP protein was expressed using a baculovirus expression system and was evaluated for its ability to elicit antibodies which may interfere with sperm-ZP interaction. The expressed **glycosylated protein**, BV55, was purified by wheat germ agglutinin lectin affinity chromatog. Antisera made in guinea pigs

searcher : m.smith 83278

immunized with BV55 (GP-.alpha.-BV55) is specific for the 55 kDa rabbit ZP protein. Indirect immunofluorescence studies indicate that GP-.alpha.-BV55 localizes to a filamentous meshwork on the surface of the ZP of isolated rabbit eggs. Immunohistochem. anal. of rabbit ovaries demonstrated that this antigen is localized within the ZP of primary and more advanced stage ovarian follicles but is not detected in primordial follicles. In addn., the 55 kDa antigen was detected in the granulosa cells of secondary stage follicles but not in the oocyte. GP-.alpha.-BV55 effectively blocked the binding of rabbit sperm to rabbit eggs in vitro. However, Fab fragments generated from GP-.alpha.-BV55 failed to block sperm binding, suggesting that the inhibitory effect of GP-.alpha.-BV55 was due to steric hindrance rather than specific blocking of a sperm receptor site. Although the Fab fragment did not inhibit sperm binding, addnl. studies demonstrated that biotinylated BV55 protein bound to rabbit sperm in the acrosomal region in a manner consistent with ligand activity in the sperm-ZP interaction, and that BV55 bound to rabbit sperm in a dose-dependent manner. These studies therefore demonstrate that antibodies against recombinant ZP proteins recognize the native intact ZP and inhibit sperm-ZP interaction. They also provide evidence that the rabbit 55 kDa ZP protein, which is the homolog of the pig **ZP3** .alpha. sperm receptor protein, has sperm receptor activity.

L8 ANSWER 3 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1995:985124 HCAPLUS

DOCUMENT NUMBER: 124:78116

TITLE: Allelic polymorphism in the hamster oviductin gene is due to a variable number of mucin-like tandem repeats

AUTHOR(S): Paquette, Yves; Merlen, Yannick; Malette, Brigitte; Bleau, Gilles

CORPORATE SOURCE: Dep. of Biochemistry, Univ. de Montreal, Montreal, PQ, Can.

SOURCE: Mol. Reprod. Dev. (1995), 42(4), 388-96
CODEN: MREDEE; ISSN: 1040-452X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Oviductins are high-mol.-wt. glycoproteins specifically secreted by the oviduct. These proteins bind to the **zona pellucida** of the ovulated oocyte and remain assocd. with the embryo during its transit in the oviduct. They may be involved in fertilization and early embryonic development. In order to explore their putative biol. function, the cDNA sequence corresponding to oviductin in the golden hamster was detd. The deduced amino acid sequence of this heavily O-**glycosylated** **protein** presents characteristics typical of mucins, including serine- or threonine-rich tandem repeats. Anal. of several cDNA clones and of genomic DNA revealed the presence of a single copy gene with 2 frequent alleles differing in the no. of repeats. Comparison with oviductin sequences from other mammals indicates a high degree of conservation amongst species, except fr the repeat region which shows divergence, notably in the no. of repeats. Based on its biochem. and genetic properties, hamster oviductin can now be classified as a secretory mucin. This concept provides a new insight in the elucidation of its biol. role: oviductin could possibly provide the oviduct and the oocyte with a protective coating ensuring normal tubal function and embryonic development.

L8 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2000 ACS

searcher : m.smith 83278

ACCESSION NUMBER: 1995:504019 HCAPLUS
 DOCUMENT NUMBER: 122:256544
 TITLE: Immunogenicity enhancement of recombinant rabbit
 55-kilodalton **zona pellucida**
protein expressed using the baculovirus
 expression system
 AUTHOR(S): Prasad, Sarvamangala V.; Mujtaba, Shiraz; Lee, Vaughan
 H.; Dunbar, Bonnie S.
 CORPORATE SOURCE: Dep. Cell Biol., Baylor Coll. Med., Houston, TX,
 77030, USA
 SOURCE: Biol. Reprod. (1995), 52(5), 1167-78
 CODEN: BIREBV; ISSN: 0006-3363
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB In the present study we have used a mol. approach to evaluate the
 immunogenicity of glycosylated and non-glycosylated recombinant rabbit
 55-kDa **zona pellucida** (ZP) **protein**. The
 55-kDa cDNA was expressed in insect (Sf9) cells through use of a
 baculovirus expression system to obtain nonfusion **glycosylated**
 recombinant ZP **protein** (BV-55). SDS-PAGE and immunoblot anal.
 demonstrated that the recombinant **protein** is expressed as two
 forms having relative mol. masses of 70 kDa and 80 kDa. Because cells
 treated with tunicamycin produce predominantly the 70-kDa form, this
 heterogeneity is presumed to be due to differential glycosylation.
 Further studies using lectin blot and immunoblot analyses showed that the
 BV-55 **protein** has both N-linked and O-linked oligosaccharides.
 However, this glycosylation is distinct from that of the native 55 kDa ZP
protein since it was not recognized by a monoclonal antibody
 assocd. with lactosaminoglycan-type carbohydrate epitopes in native ZP
 proteins. Immunogenicity studies demonstrated that antibodies against the
 BV-55 **protein** are developed by female rabbits and guinea pigs
 and that these antibodies recognize epitopes assocd. with native,
 enzyme-deglycosylated as well as nonglycosylated recombinant forms of the
 rabbit 55-kDa ZP **protein**. In contrast, recombinant
protein expressed in bacteria did not elicit antibodies in either
 rabbits or guinea pigs. These results demonstrate that expression of the
 55-kDa recombinant **protein** in the baculovirus expression system
 enhances its immunogenicity.

L8 ANSWER 5 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1994:127987 HCAPLUS
 DOCUMENT NUMBER: 120:127987
 TITLE: Characterization of two glycosylated boar
 spermadhesins
 AUTHOR(S): Calvete, Juan Jose; Solis, Dolores; Sanz, Libia;
 Diaz-Maurino, Teresa; Schaefer, Wolfram; Mann,
 Karlheinz; Topfer-Petersen, Edda
 CORPORATE SOURCE: Inst. Reproduktionsmed., Tieraerztl. Hochsch.
 Hannover, Hannover, D-30599, Germany
 SOURCE: Eur. J. Biochem. (1993), 218(2), 719-25
 CODEN: EJBCAI; ISSN: 0014-2956
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB Boar spermadhesins AQN-1, AQN-3 and AWN form a recently described
protein family, synthesized by the sexual accessory glands, and
 become assocd. with the sperm head upon ejaculation. They contain 109-133

searcher : m.smith 83278

amino acid residues, two conserved disulfide bridges, are not glycosylated, and have 40-60% primary structure identity. These boar polypeptides are multifunctional proteins, which possess heparin-, serine-protease-inhibitor- and/or **zona-pellucida** -glycoprotein-binding capability and have, therefore, been implicated in sperm capacitation and sperm-oocyte attachment. AQN-2 (18-20 kDa), however, is unique among boar spermadhesins in that it is the only member of the family which is known to be glycosylated and which possesses weak **zona-pellucida**-binding but not seminal-plasma-inhibitor-binding ability. In this study the authors report the structural and functional characterization of the two glycoproteins contained in the AQN-2 fraction. One component is identical with PSP-I, a major porcine seminal plasma **protein** whose function has not yet been identified, while the second **protein** is a **glycosylated** isoform of AQN-3. Here the authors show that the inability of the glycosylated boar spermadhesins to bind seminal-plasma protease inhibitors as well as the weak binding of glycosylated AQN-3 to **zona pellucida** glycoproteins is due to the presence of the oligosaccharide chain on a conserved asparagine residue. This indicates that modification of a spermadhesin polypeptide framework may serve to modulate its ligand-binding capabilities.

L8 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1993:468907 HCAPLUS

DOCUMENT NUMBER: 119:68907

TITLE: Identification of a region of mouse pellucida glycoprotein mZP3 that possesses sperm receptor activity

AUTHOR(S): Rosiere, Thomas K.; Wassarman, Paul M.

CORPORATE SOURCE: Roche Inst. Mol. Biol., Roche Research Cent., Nutley, NJ, 07110, USA

SOURCE: Dev. Biol. (1992), 154(2), 309-17
CODEN: DEBIAO; ISSN: 0012-1606

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Purified, radioiodinated mouse zona pellucida glycoprotein **ZP3** (mZP3) was digested by either papain or V8 protease, and the glycopeptides produced were fractionated by HPLC and assayed for sperm receptor activity in vitro. Each proteolytic digest of mZP3 contained a heavily **glycosylated peptide**, .apprx.55,000 apparent Mr, that exhibited sperm receptor activity in vitro. To det. the region of mZP3 polypeptide from which the active glycopeptides were derived, Western gel immunoblotting, employing an antiserum directed against a specific mZP3 peptide epitope, and automated N-terminal amino acid sequencing were employed. The active glycopeptides produced by digestion of mZP3 with either papain or V8 protease are derived from the same region of the C-terminal half of the mZP3 polypeptide. These and other findings are discussed in terms of mZP3 structure and function.

L8 ANSWER 7 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1992:647377 HCAPLUS

DOCUMENT NUMBER: 117:247377

TITLE: Purification and partial characterization of the 17 kDa sperm coating protein from boar seminal plasma

AUTHOR(S): Moos, Jiri; Veselsky, Leopold; Peknicova, Jana; Drahorad, Josef

searcher : m.smith 83278

CORPORATE SOURCE: Inst. Mol. Genet., Czech. Acad. Sci., Prague, Czech.
 SOURCE: Mol. Reprod. Dev. (1992), 33(2), 165-71
 CODEN: MREDEE; ISSN: 1040-452X

DOCUMENT TYPE: Journal
 LANGUAGE: English

AB Sperm coating proteins of 16, 17, and 19 kDa have been purified from boar seminal plasma. The 17 kDa **protein** has been identified as an antigen recognized by monoclonal antibody ACR.3 and is thus identical to low mol. mass **zona pellucida binding protein** from boar spermatozoa (Moos, J., et al., 1990). The 17 and 19 kDa **proteins** are **glycosylated** and tend to form hetero-complexes. The 17 kDa ACR.3 antigen is sequentially released from the sperm cell surface during capacitation and, after induction of the acrosome reaction, the 16 kDa form was also obsd. Immunocytochem. studies on boar reproductive tissues have suggested that the seminal vesicle epithelium may be the source of these proteins.

L8 ANSWER 8 OF 9 HCAPLUS COPYRIGHT 2000 ACS

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 DOCUMENT NUMBER: 109:34792
 TITLE: Monoclonal antibodies specific for an oviductal component associated with the hamster zona pellucida
 AUTHOR(S): St-Jacques, Sylvie; Bleau, Gilles
 CORPORATE SOURCE: Dep. Obstet. Gynecol., Univ. Montreal, Montreal, PQ, Can.
 SOURCE: J. Reprod. Immunol. (1988), 12(4), 247-61
 CODEN: JRIMDR; ISSN: 0165-0378

DOCUMENT TYPE: Journal
 LANGUAGE: English

AB Five monoclonal antibodies (MAbs) were produced against oviductal **zona pellucida** (ZP) of the hamster. They were purified from ascitic fluid by HPLC on hydroxylapatite and anion-exchange columns. All 5 MAbs reacted selectively with oviductal ZP and oviductal secretions; no binding was obsd. to intra-ovarian ZP. A study of the tissue specificity, as evaluated by indirect immunofluorescence, revealed the binding of all of these Abs only to the oviduct and, to a lesser extent, to the uterus. A cytosolic fraction from hamster oviduct was subjected to SDS-PAGE under reducing conditions and electro-transfer to a nitrocellulose membrane; immunoenzymic staining showed a reaction with a polydispersed oviductal component of high mol. wt. (.apprx.200,000). The native antigen has a mol. wt. >400,000 as detd. by mol. sieve chromatog. Thus, an oviductal antigen is added to the hamster ZP during its transit through the oviduct. This antigen, called oviductin, is a heavily **glycosylated protein** of high mol. wt.

L8 ANSWER 9 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1980:161203 HCAPLUS
 DOCUMENT NUMBER: 92:161203
 TITLE: Synthesis of zona pellucida proteins by denuded and follicle-enclosed mouse oocytes during culture in vitro
 AUTHOR(S): Bleil, Jeffrey D.; Wassarman, Paul M.
 CORPORATE SOURCE: Dep. Biol. Chem., Harvard Med. Sch., Boston, MA, 02115, USA
 SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1980), 77(2), 1029-33
 CODEN: PNASA6; ISSN: 0027-8424

searcher : m.smith 83278

DOCUMENT TYPE: Journal
LANGUAGE: English

AB To det. whether the zona pellucida originates from the oocyte, surrounding follicle cells, or both, denuded and follicle-enclosed mouse oocytes at various stages of growth were isolated and cultured in vitro in the presence of either methionine-35S or fucose-3H to det. the site of synthesis of the 3 recently identified zona pellucida proteins, ZP1, ZP2, and ZP3. Approx. 1.5% of the methionine-35S, and as much as 45% of the fucose-3H, that was incorporated into TCA-insol. material by denuded or follicle-enclosed oocytes during a 12-h culture period was assocd. with zonae pellucidae removed from the cultured oocytes. Incorporation of methionine-35S into zona pellucida proteins was depressed to <1/50th when denuded oocytes were cultured in the presence of puromycin, and secretion of zona pellucida proteins by denuded oocytes was demonstrated by pulse-chase expts. Na dodecyl sulfate-polyacrylamide gel electrophoresis of methionine-35S- and fucose-3H-labeled proteins present in oocytes, zonae pellucidae, and follicle cells revealed that denuded oocytes synthesize and secrete zona pellucida proteins, whereas no evidence was obtained to suggest that follicle cells synthesize these proteins. Denuded oocytes, ranging in diam. from 48 to 68 .mu., incorporated both methionine-35S and fucose-3H into zona pellucida proteins during culture in vitro, whereas zonae pellucidae removed from fully-grown oocytes (85 .mu.) were not radiolabeled to a significant extent. After culture of denuded or follicle-enclosed oocytes for 12 h, >95% of the fucose-3H incorporated into oocyte proteins was found in ZP1, ZP2, and ZP3, indicating that zona pellucida proteins are the major class of **proteins glycosylated** during oocyte growth. Apparently, the zona pellucida originates from the mammalian oocyte itself, rather than from the surrounding follicle cells.

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        RHZP?
L2      11 SEA FILE=REGISTRY HUMAN(L) ZP3
L3      1 SEA FILE=REGISTRY "GLYCOSYLATED PROTEINS"/CN
L4      185 SEA FILE=HCAPLUS L1 OR L2 OR (HUMAN(L) (ZONA(L) PELLUCIDA(L)
        PROTEIN OR ZP3)) OR RHZP?
L5      2170 SEA FILE=HCAPLUS GLYCOSYLATED(2A) (PROTEIN? OR PEPTIDE?) OR L3
L6      2 SEA FILE=HCAPLUS L4 AND L5
L7      746 SEA FILE=HCAPLUS (ZONA(2A) PELLUCIDA(L) PROTEIN OR ZP3) OR
        RHZP?
L8      9 SEA FILE=HCAPLUS (L5 AND L7) NOT L6
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=> d ibib abs hitrn l6 1-2

L6 ANSWER 1 OF 2 HCAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:226653 HCAPLUS
DOCUMENT NUMBER: 131:98261
TITLE: Cloning and characterization of a zona pellucida 3
cDNA from a marsupial, the brushtail possum
Trichosurus vulpecula
AUTHOR(S): McCartney, Carmen A.; Mate, Karen E.
CORPORATE SOURCE: Cooperative Research Centre for Conservation and

M. Smith 308-3278

SOURCE: Management of Marsupials, School of Biological Sciences, Macquarie University, 2109, Australia
 Zygote (1999), 7(1), 1-9
 CODEN: ZYGOEB; ISSN: 0967-1994
 PUBLISHER: Cambridge University Press
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB The mammalian **zona pellucida** (ZP) is an extracellular glycoprotein coat that plays vital roles throughout fertilization and preimplantation development. Like that of eutherian mammals the brushtail possum ZP is composed of three **glycosylated proteins** of 137 kDa, 92 kDa and 62 kDa. The 62 kDa **protein** is a **ZP3** ortholog based on its nucleotide and deduced amino acid sequence. The brushtail possum **ZP3** cDNA isolated in this study is 1305 nucleotides with an open reading frame encoding a 422 amino acid peptide of 45.7 kDa. Possum **ZP3** has a 46% amino acid identity with eutherian **ZP3** and shares similar structural characteristics including 12 conserved cysteine residues, N-linked glycosylation sites and hydrophobic regions. Like **human** and rabbit ZP1 an altered furin cleavage site upstream of the C-terminal hydrophobic domain also occurs in possum **ZP3** (S-R-K-R), suggestive of processing by a furin-related endoprotease. Expression of brushtail possum **ZP3** is limited to the ovary. Characterization of brushtail possum **ZP3** will enable examn. of its functional role in marsupial fertilization and its effectiveness as an immunocontraceptive agent.

L6 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1998:404617 HCAPLUS
 DOCUMENT NUMBER: 129:187090
 TITLE: Modeling human sperm-egg interactions in vitro: signal transduction pathways regulating the acrosome reaction
 AUTHOR(S): Benoff, Susan
 CORPORATE SOURCE: Department of Obstetrics and Gynecology, North Shore University Hospital-New York University Medical College, Manhasset, NY, 11030, USA
 SOURCE: Mol. Hum. Reprod. (1998), 4(5), 453-471
 CODEN: MHREFD; ISSN: 1360-9947
 PUBLISHER: Oxford University Press
 DOCUMENT TYPE: Journal; General Review
 LANGUAGE: English

AB A review with many refs. Recent advances in characterizing sperm surface receptors and ion channels, when combined with the rapidly expanding knowledge of interactions among second messenger systems in somatic cells, permit formulation of a tentative mol. mechanism for the regulation of the **human** sperm acrosome reaction. As spermatozoa pass through the cumulus mass, progesterone binds to its sperm surface receptor, alkalinizes the sperm head cytosol and potentiates changes in intracellular ionized calcium. Primary binding of spermatozoa to egg involves receptors for mannosyl, N-acetylglucosaminyl and, possibly, fucosyl residues of the **glycosylated zona protein, ZP3**. These receptors aggregate on multivalent ligand binding, migrate to the equatorial region along an actin filament network formed between the plasma and acrosomal membranes during capacitation, and activate a G protein/protein kinase A/protein kinase C second messenger

system and a secondary proteolysis signal. Binding of a receptor tyrosine kinase to **ZP3** amino acid residues simultaneous with the sugar recognition event triggers tyrosine phosphorylation signalling. All signals combine to open a voltage-dependent calcium channel. The resulting elevated calcium signal depolymerizes the inter-membrane actin network and activates phospholipases, leading to an acrosome reaction.

=> d ibib abs hitrn 18 1-9

L8 ANSWER 1 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1999:608306 HCAPLUS
DOCUMENT NUMBER: 131:335874
TITLE: Expression of a Recombinant Porcine Zona Pellucida Glycoprotein ZP1 in Mammalian Cells
AUTHOR(S): Tsubamoto, Hiroshi; Yamasaki, Noriyuki; Hasegawa, Akiko; Koyama, Koji
CORPORATE SOURCE: Department of Obstetrics and Gynecology, Hyogo College of Medicine, Nishinomiya, 663-8501, Japan
SOURCE: Protein Expression Purif. (1999), 17(1), 8-15
CODEN: PEXPEJ; ISSN: 1046-5928
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Porcine zona pellucida glycoprotein (pZP1) is a good candidate for a contraceptive vaccine. For the purpose of producing glycosylated pZP1, several types of recombinant pZP1 proteins were produced in mammalian cell lines. In the first expt., a minigene encoding pZP1 (681 amino acids) was designed for insertion into an expression vector and then transfected to three cell lines (293T, CHO-K1, and LLC-PK1). The resulting recombinant **proteins** were highly **glycosylated** and were localized in the cytoplasm. To produce a secretory type of recombinant pZP1, in the second expt., a cDNA coding for pZP1 excluding a putative transmembrane region and a smaller cDNA coding for 1-198 amino acid residues of pZP1 were designed to produce fusion proteins with the human IgG1 heavy chain. The resultant recombinant proteins were secreted into the supernatant from both transfected cell cultures. Recombinant secretory proteins are useful because of their simple affinity purifn. (c) 1999 Academic Press.

L8 ANSWER 2 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1996:205023 HCAPLUS
DOCUMENT NUMBER: 124:312986
TITLE: Evaluating **zona pellucida** structure and function using antibodies to rabbit 55 kDa ZP **protein** expressed in baculovirus expression system
AUTHOR(S): Prasad, Sarvamangala V.; Wilkins, Brendan; Skinner, Sheri M.; Dunbar, Bonnie S.
CORPORATE SOURCE: Dep. Cell Biol., Baylor Coll. Med., Houston, TX, 77030, USA
SOURCE: Mol. Reprod. Dev. (1996), 43(4), 519-29
CODEN: MREDEE; ISSN: 1040-452X
DOCUMENT TYPE: Journal

M. Smith 308-3278

LANGUAGE: English

AB A cDNA encoding the rabbit 55 kDa ZP protein was expressed using a baculovirus expression system and was evaluated for its ability to elicit antibodies which may interfere with sperm-ZP interaction. The expressed **glycosylated protein**, BV55, was purified by wheat germ agglutinin lectin affinity chromatog. Antisera made in guinea pigs immunized with BV55 (GP-.alpha.-BV55) is specific for the 55 kDa rabbit ZP protein. Indirect immunofluorescence studies indicate that GP-.alpha.-BV55 localizes to a filamentous meshwork on the surface of the ZP of isolated rabbit eggs. Immunohistochem. anal. of rabbit ovaries demonstrated that this antigen is localized within the ZP of primary and more advanced stage ovarian follicles but is not detected in primordial follicles. In addn., the 55 kDa antigen was detected in the granulosa cells of secondary stage follicles but not in the oocyte. GP-.alpha.-BV55 effectively blocked the binding of rabbit sperm to rabbit eggs in vitro. However, Fab fragments generated from GP-.alpha.-BV55 failed to block sperm binding, suggesting that the inhibitory effect of GP-.alpha.-BV55 was due to steric hindrance rather than specific blocking of a sperm receptor site. Although the Fab fragment did not inhibit sperm binding, addnl. studies demonstrated that biotinylated BV55 protein bound to rabbit sperm in the acrosomal region in a manner consistent with ligand activity in the sperm-ZP interaction, and that BV55 bound to rabbit sperm in a dose-dependent manner. These studies therefore demonstrate that antibodies against recombinant ZP proteins recognize the native intact ZP and inhibit sperm-ZP interaction. They also provide evidence that the rabbit 55 kDa ZP protein, which is the homolog of the pig **ZP3** .alpha. sperm receptor protein, has sperm receptor activity.

L8 ANSWER 3 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1995:985124 HCAPLUS

DOCUMENT NUMBER: 124:78116

TITLE: Allelic polymorphism in the hamster oviductin gene is due to a variable number of mucin-like tandem repeats

AUTHOR(S): Paquette, Yves; Merlen, Yannick; Malette, Brigitte; Bleau, Gilles

CORPORATE SOURCE: Dep. of Biochemistry, Univ. de Montreal, Montreal, PQ, Can.

SOURCE: Mol. Reprod. Dev. (1995), 42(4), 388-96

CODEN: MREDEE; ISSN: 1040-452X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Oviductins are high-mol.-wt. glycoproteins specifically secreted by the oviduct. These proteins bind to the **zona pellucida** of the ovulated oocyte and remain assocd. with the embryo during its transit in the oviduct. They may be involved in fertilization and early embryonic development. In order to explore their putative biol. function, the cDNA sequence corresponding to oviductin in the golden hamster was detd. The deduced amino acid sequence of this heavily O-**glycosylated protein** presents characteristics typical of mucins, including serine- or threonine-rich tandem repeats. Anal. of several cDNA clones and of genomic DNA revealed the presence of a single copy gene with 2 frequent alleles differing in the no. of repeats. Comparison with oviductin sequences from other mammals indicates a high degree of conservation amongst species, except for the repeat region which shows

divergence, notably in the no. of repeats. Based on its biochem. and genetic properties, hamster oviductin can now be classified as a secretory mucin. This concept provides a new insight in the elucidation of its biol. role: oviductin could possibly provide the oviduct and the oocyte with a protective coating ensuring normal tubal function and embryonic development.

L8 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1995:504019 HCAPLUS

DOCUMENT NUMBER: 122:256544

TITLE: Immunogenicity enhancement of recombinant rabbit
55-kilodalton **zona pellucida**
protein expressed using the baculovirus
expression system

AUTHOR(S): Prasad, Sarvamangala V.; Mujtaba, Shiraz; Lee, Vaughan
H.; Dunbar, Bonnie S.

CORPORATE SOURCE: Dep. Cell Biol., Baylor Coll. Med., Houston, TX,
77030, USA

SOURCE: Biol. Reprod. (1995), 52(5), 1167-78
CODEN: BIREBV; ISSN: 0006-3363

DOCUMENT TYPE: Journal

LANGUAGE: English

AB In the present study we have used a mol. approach to evaluate the immunogenicity of glycosylated and non-glycosylated recombinant rabbit 55-kDa **zona pellucida** (ZP) **protein**. The 55-kDa cDNA was expressed in insect (Sf9) cells through use of a baculovirus expression system to obtain nonfusion **glycosylated** recombinant ZP **protein** (BV-55). SDS-PAGE and immunoblot anal. demonstrated that the recombinant **protein** is expressed as two forms having relative mol. masses of 70 kDa and 80 kDa. Because cells treated with tunicamycin produce predominantly the 70-kDa form, this heterogeneity is presumed to be due to differential glycosylation. Further studies using lectin blot and immunoblot analyses showed that the BV-55 **protein** has both N-linked and O-linked oligosaccharides. However, this glycosylation is distinct from that of the native 55 kDa ZP **protein** since it was not recognized by a monoclonal antibody assocd. with lactosaminoglycan-type carbohydrate epitopes in native ZP proteins. Immunogenicity studies demonstrated that antibodies against the BV-55 **protein** are developed by female rabbits and guinea pigs and that these antibodies recognize epitopes assocd. with native, enzyme-deglycosylated as well as nonglycosylated recombinant forms of the rabbit 55-kDa ZP **protein**. In contrast, recombinant **protein** expressed in bacteria did not elicit antibodies in either rabbits or guinea pigs. These results demonstrate that expression of the 55-kDa recombinant **protein** in the baculovirus expression system enhances its immunogenicity.

L8 ANSWER 5 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1994:127987 HCAPLUS

DOCUMENT NUMBER: 120:127987

TITLE: Characterization of two glycosylated boar
spermadhesins

AUTHOR(S): Calvete, Juan Jose; Solis, Dolores; Sanz, Libia;
Diaz-Maurino, Teresa; Schaefer, Wolfram; Mann,

Karlheinz; Topfer-Petersen, Edda
 CORPORATE SOURCE: Inst. Reproduktionsmed., Tieraerztl. Hochsch.
 Hannover, Hannover, D-30599, Germany
 SOURCE: Eur. J. Biochem. (1993), 218(2), 719-25
 CODEN: EJBCAI; ISSN: 0014-2956
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB Boar spermadhesins AQN-1, AQN-3 and AWN form a recently described **protein** family, synthesized by the sexual accessory glands, and become assocd. with the sperm head upon ejaculation. They contain 109-133 amino acid residues, two conserved disulfide bridges, are not glycosylated, and have 40-60% primary structure identity. These boar polypeptides are multifunctional proteins, which possess heparin-, serine-protease-inhibitor- and/or **zona-pellucida** -glycoprotein-binding capability and have, therefore, been implicated in sperm capacitation and sperm-oocyte attachment. AQN-2 (18-20 kDa), however, is unique among boar spermadhesins in that it is the only member of the family which is known to be glycosylated and which possesses weak **zona-pellucida**-binding but not seminal-plasma-inhibitor-binding ability. In this study the authors report the structural and functional characterization of the two glycoproteins contained in the AQN-2 fraction. One component is identical with PSP-I, a major porcine seminal plasma **protein** whose function has not yet been identified, while the second **protein** is a **glycosylated** isoform of AQN-3. Here the authors show that the inability of the glycosylated boar spermadhesins to bind seminal-plasma protease inhibitors as well as the weak binding of glycosylated AQN-3 to **zona pellucida** glycoproteins is due to the presence of the oligosaccharide chain on a conserved asparagine residue. This indicates that modification of a spermadhesin polypeptide framework may serve to modulate its ligand-binding capabilities.

L8 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2000 ACS
 ACCESSION NUMBER: 1993:468907 HCAPLUS
 DOCUMENT NUMBER: 119:68907
 TITLE: Identification of a region of mouse pellucida glycoprotein mZP3 that possesses sperm receptor activity
 AUTHOR(S): Rosiere, Thomas K.; Wassarman, Paul M.
 CORPORATE SOURCE: Roche Inst. Mol. Biol., Roche Research Cent., Nutley, NJ, 07110, USA
 SOURCE: Dev. Biol. (1992), 154(2), 309-17
 CODEN: DEBIAO; ISSN: 0012-1606
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB Purified, radioiodinated mouse zona pellucida glycoprotein **ZP3** (mZP3) was digested by either papain or V8 protease, and the glycopeptides produced were fractionated by HPLC and assayed for sperm receptor activity in vitro. Each proteolytic digest of mZP3 contained a heavily **glycosylated peptide**, .apprx.55,000 apparent Mr, that exhibited sperm receptor activity in vitro. To det. the region of mZP3 polypeptide from which the active glycopeptides were derived, Western gel immunoblotting, employing an antiserum directed against a specific mZP3 peptide epitope, and automated N-terminal amino acid sequencing were

employed. The active glycopeptides produced by digestion of mZP3 with either papain or V8 protease are derived from the same region of the C-terminal half of the mZP3 polypeptide. These and other findings are discussed in terms of mZP3 structure and function.

L8 ANSWER 7 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1992:647377 HCAPLUS
DOCUMENT NUMBER: 117:247377
TITLE: Purification and partial characterization of the 17 kDa sperm coating protein from boar seminal plasma
AUTHOR(S): Moos, Jiri; Veselsky, Leopold; Peknicova, Jana; Drahorad, Josef
CORPORATE SOURCE: Inst. Mol. Genet., Czech. Acad. Sci., Prague, Czech.
SOURCE: Mol. Reprod. Dev. (1992), 33(2), 165-71
CODEN: MREDEE; ISSN: 1040-452X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Sperm coating proteins of 16, 17, and 19 kDa have been purified from boar seminal plasma. The 17 kDa **protein** has been identified as an antigen recognized by monoclonal antibody ACR.3 and is thus identical to low mol. mass **zona pellucida binding protein** from boar spermatozoa (Moos, J., et al., 1990). The 17 and 19 kDa **proteins** are **glycosylated** and tend to form hetero-complexes. The 17 kDa ACR.3 antigen is sequentially released from the sperm cell surface during capacitation and, after induction of the acrosome reaction, the 16 kDa form was also obsd. Immunocytochem. studies on boar reproductive tissues have suggested that the seminal vesicle epithelium may be the source of these proteins.

L8 ANSWER 8 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1988:434792 HCAPLUS
DOCUMENT NUMBER: 109:34792
TITLE: Monoclonal antibodies specific for an oviductal component associated with the hamster zona pellucida
AUTHOR(S): St-Jacques, Sylvie; Bleau, Gilles
CORPORATE SOURCE: Dep. Obstet. Gynecol., Univ. Montreal, Montreal, PQ, Can.
SOURCE: J. Reprod. Immunol. (1988), 12(4), 247-61
CODEN: JRIMDR; ISSN: 0165-0378
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Five monoclonal antibodies (MAbs) were produced against oviductal **zona pellucida** (ZP) of the hamster. They were purified from ascitic fluid by HPLC on hydroxylapatite and anion-exchange columns. All 5 MAbs reacted selectively with oviductal ZP and oviductal secretions; no binding was obsd. to intra-ovarian ZP. A study of the tissue specificity, as evaluated by indirect immunofluorescence, revealed the binding of all of these Abs only to the oviduct and, to a lesser extent, to the uterus. A cytosolic fraction from hamster oviduct was subjected to SDS-PAGE under reducing conditions and electro-transfer to a nitrocellulose membrane; immunoenzymic staining showed a reaction with a polydispersed oviductal component of high mol. wt. (.apprx.200,000). The native antigen has a mol. wt. >400,000 as detd. by mol. sieve chromatog. Thus, an oviductal antigen is added to the hamster ZP during its transit

through the oviduct. This antigen, called oviductin, is a heavily **glycosylated protein** of high mol. wt.

L8 ANSWER 9 OF 9 HCAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1980:161203 HCAPLUS

DOCUMENT NUMBER: 92:161203

TITLE: Synthesis of zona pellucida proteins by denuded and follicle-enclosed mouse oocytes during culture in vitro

AUTHOR(S): Bleil, Jeffrey D.; Wassarman, Paul M.

CORPORATE SOURCE: Dep. Biol. Chem., Harvard Med. Sch., Boston, MA, 02115, USA

SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1980), 77(2), 1029-33

CODEN: PNASA6; ISSN: 0027-8424

DOCUMENT TYPE: Journal

LANGUAGE: English

AB To det. whether the zona pellucida originates from the oocyte, surrounding follicle cells, or both, denuded and follicle-enclosed mouse oocytes at various stages of growth were isolated and cultured in vitro in the presence of either methionine-35S or fucose-3H to det. the site of synthesis of the 3 recently identified zona pellucida proteins, ZP1, ZP2, and **ZP3**. Approx. 1.5% of the methionine-35S, and as much as 45% of the fucose-3H, that was incorporated into TCA-insol. material by denuded or follicle-enclosed oocytes during a 12-h culture period was assocd. with zonae pellucidae removed from the cultured oocytes. Incorporation of methionine-35S into zona pellucida proteins was depressed to <1/50th when denuded oocytes were cultured in the presence of puromycin, and secretion of zona pellucida proteins by denuded oocytes was demonstrated by pulse-chase expts. Na dodecyl sulfate-polyacrylamide gel electrophoresis of methionine-35S- and fucose-3H-labeled proteins present in oocytes, zonae pellucidae, and follicle cells revealed that denuded oocytes synthesize and secrete zona pellucida proteins, whereas no evidence was obtained to suggest that follicle cells synthesize these proteins. Denuded oocytes, ranging in diam. from 48 to 68 .mu., incorporated both methionine-35S and fucose-3H into zona pellucida proteins during culture in vitro, whereas zonae pellucidae removed from fully-grown oocytes (85 .mu.) were not radiolabeled to a significant extent. After culture of denuded or follicle-enclosed oocytes for 12 h, >95% of the fucose-3H incorporated into oocyte proteins was found in ZP1, ZP2, and **ZP3**, indicating that zona pellucida proteins are the major class of **proteins glycosylated** during oocyte growth. Apparently, the zona pellucida originates from the mammalian oocyte itself, rather than from the surrounding follicle cells.

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File 155:MEDLINE(R) 1966-2001/Aug W2
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Set	Items	Description
S1	300	(GLYCOPEPTIDE? OR GLYCOPROTEIN?) (S) (ZP(W)3 OR ZP(W)III OR ZONA(W)PELLUCIDA OR HZP3 OR MZP3 OR ZP3) (S) OVAR?(S)SPERM?
S2	71	RD (unique items)
S3	47	S2 NOT PY=(2001 OR 2000 OR 1999 OR 1998)

?t s3/3 ab/1-47

3/AB/1 (Item 1 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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09658730 98119365 PMID: 9459277

Prospects of zona pellucida glycoproteins as immunogens for contraceptive vaccine.

Gupta SK; Jethanandani P; Afzalpurkar A; Kaul R; Santhanam R
Gamete Antigen Laboratory, National Institute of Immunology, Aruna Asaf
Ali Marg, New Delhi, India. GA@nii.ernet.in

Human reproduction update (ENGLAND) Jul-Aug 1997, 3 (4) p311-24,
ISSN 1355-4786 Journal Code: CUH

Comment in Hum Reprod Update. 1997 Jul-Aug;3(4) 299-300

Languages: ENGLISH

Document type: Journal Article; Review; Review, Tutorial

Record type: Completed

The zona pellucida (ZP) surrounding a mammalian oocyte mediates the initial recognition and binding of spermatozoon to oocyte in a relatively species-specific manner and plays an important role in the subsequent activation events during the fertilization process. The ZP comprises three biochemically and immunologically distinct glycoproteins termed ZP1, ZP2 and ZP3. The critical role of ZP glycoproteins in reproduction together with their tissue-specific nature have led to their being considered as potential candidate antigens for immunocontraception. Immunization of females with ZP glycoproteins leads to a block of fertility in several animal models. However, it is invariably associated with either a transient or an irreversible alteration in the cyclicity, hormonal profile and follicular development in the ovary. To overcome these problems, attempts are being made to delineate relevant 'B' cell epitopes on ZP proteins so as to design immunocontraceptive vaccines based on synthetic peptides devoid of oophoritogenic 'T' cell epitopes. Monoclonal antibodies capable of inhibiting the gamete interaction are being employed to delineate such regions. Additionally, DNA-recombinant technology has made it feasible to obtain, in reasonably large quantities, the ZP glycoproteins from human and non-human primates. Availability of sequence information of these zona proteins and the availability of recombinant antigens (devoid of other ovarian -associated proteins) will further help in understanding more precisely their functions during fertilization and make it feasible to undertake immunization studies to determine their prospects as immunogens for fertility regulation.

3/AB/2 (Item 2 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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09611707 98068099 PMID: 9404288

Characteristics of an oviductal glycoprotein and its potential role in fertility control.

Verhage HG; Fazleabas AT; Mavrogianis PA; O'Day-Bowman MB; Schmidt A; Arias EB; Jaffe RC

Department of Obstetrics and Gynecology, University of Illinois at Chicago 60612-7313, USA.

Journal of reproduction and fertility (ENGLAND) 1997, 51 p217-26,

ISSN 0449-3087 Journal Code: JWR

Contract/Grant No.: HD20571, HD, NICHD

Languages: ENGLISH

Document type: Journal Article; Review; Review, Tutorial

Record type: Completed

At the time of ovulation the lining epithelium of the mammalian oviduct consists of columnar ciliated and secretory cells. These mature cells are dependent on ovarian steroids in carnivores. Oestradiol induces differentiation of these cells and maintains their mature functional state, and progesterone induces dedifferentiation. The secretory cells synthesize and secrete an oestrogen-dependent high molecular weight glycoprotein. The cDNAs encoding oviductal glycoproteins from several species have been sequenced and show high similarity. The human cDNA hybridized with a single message on northern blots of total oviduct RNA obtained from oestradiol-treated cats (about 2.3 kb) and dogs (about 2.1 kb). This glycoprotein is the major nonserum protein present in the oviductal lumen at the time of ovulation, fertilization and early embryonic development. The glycoproteins associate with the zona pellucida of oviductal eggs in all species studied to date. Recent studies suggest that the bovine

glycoprotein facilitates sperm capacitation and significantly increases the ability of bovine spermatozoa to fertilize bovine oocytes in vitro, that the hamster glycoprotein increases the sperm penetration rate of the zona pellucida by three times and that the human glycoprotein increases sperm binding to the zona pellucida by three times. All of the evidence for a biological function for this glycoprotein is derived from studies performed in several different species at reproductive stages before fertilization. The biological actions of this glycoprotein suggest a potential role for the glycoprotein in fertility control. Specifically, purified or recombinant glycoprotein may improve success in IVF procedures by enhancing binding of spermatozoa to the zona pellucida and improving fertilization rates. The glycoprotein may also be a potential immunocontraceptive target since antibodies generated against the oviductal glycoprotein may prevent fertilization by preventing binding of spermatozoa to the zona pellucida .

3/AB/3 (Item 3 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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09564356 97410958 PMID: 9266007

Identification of epitopes of monoclonal antibodies to porcine zona pellucida 3 beta glycoprotein, a homologue of the mouse/human sperm receptor.

Afzalpurkar A; Gupta SK
Gamete Antigen Laboratory, National Institute of Immunology, New Delhi, India.

American journal of reproductive immunology (DENMARK) Jul 1997, 38

(1) p26-32, ISSN 1046-7408 Journal Code: AEZ

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

PROBLEM: Immunization with zona pellucida (ZP) glycoproteins leads to a block in fertility with a variable degree of ovarian dysfunction. To avoid autoimmune oophoritis, synthetic peptides corresponding to B cell epitope(s) and devoid of oophoritogenic T cell epitopes as immunogens have been proposed. The main objective of the present study is to define the epitopes recognized by monoclonal antibodies (mAbs) generated against porcine ZP3 beta, a homologue of the designated primary sperm receptor in mice and humans. METHODS: A multipin synthetic peptides approach has been used to map the epitopes recognized by mAbs. Dodecapeptides with an overlap of 6 amino acids corresponding to a precursor pZP3 beta-deduced amino acid sequence (excluding the signal sequence) were synthesized on polypropylene pins and were tested for their reactivity with mAbs by enzyme-linked immunoadsorbent assay (ELISA). The ability of synthetic peptides corresponding to the identified epitopes to inhibit the binding of mAbs to pZP3 beta in a competitive inhibition ELISA was investigated to confirm the above findings. RESULTS: Reactivity of the mAbs with the pin-bound peptides in ELISA-identified epitopes for mAb-451 to EEKLVF (166-171) and mAb-462/470 to FKAPRP (250-255) amino acid residues. mAb-30 recognized QPVWQDEGQRLR (23-34) and VICRCC (316-321) amino acid residues. Competitive inhibition with synthetic peptides encompassing the motifs corresponding to 23-34 and 316-321 for binding of mAb-30 to pZP3 beta revealed the epitopic domain to be 23-34 amino acids. Synthesis of overlapping octapeptides further identified WQDE as the minimum motif for binding of mAb-30, and the replacement of one amino acid at a time with

glycine revealed tryptophan as the critical residue. CONCLUSIONS: Collectively, these results describe peptide epitopes that will help in the design of an immunocontraceptive vaccine based on synthetic peptides corresponding to pZP3 beta or its homologues in other species.

3/AB/4 (Item 4 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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09341975 97156170 PMID: 9002630
Antibody responses and infertility in mice following oral immunization with attenuated Salmonella typhimurium expressing recombinant murine ZP3. Zhang X; Lou YH; Koopman M; Doggett T; Tung KS; Curtiss R
Department of Biology, Washington University, St. Louis, Missouri 63130, USA.

Biology of reproduction (UNITED STATES) Jan 1997, 56 (1) p33-41,
ISSN 0006-3363 Journal Code: A3W
Erratum in Biol Reprod 1997 Apr;56(4) 1069
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

Ovarian ZP3, the primary sperm receptor, is a major glycoprotein of mouse zona pellucida (ZP). Because antibodies raised against ZP3 block sperm-egg interaction, ZP3 has been considered a candidate immunogen in the development of a contraceptive vaccine. This study explored the possibility of using an attenuated Salmonella typhimurium vaccine strain expressing recombinant ZP3 to elicit an antibody response and infertility in mice. A cDNA sequence generated by the polymerase chain reaction encoding 342 amino acid residues (23-364) of the mouse (m)ZP3 was cloned into an Asd+ vector. An avirulent Salmonella vaccine strain stably expressed the ZP3 polypeptide and colonized the internal organs of mice after oral inoculation. Oral immunization of female BALB/c mice with the recombinant Salmonella vaccine strain expressing mZP3 induced significant levels of anti-native ZP IgG antibodies in serum and IgA antibodies in vaginal secretions. The IgG antibodies thus induced also bound to ZP in vivo. When mated with males, 3 of 6 females immunized with the recombinant Salmonella were infertile. In contrast, none of the mice that received Salmonella containing the vector plasmid produced antibodies to ZP and all were fertile. No ovarian inflammation was observed in the immunized mice at autopsy. The results suggest a potential oral contraceptive vaccine to control populations of rodent vectors of disease and to induce reversible infertility in humans.

3/AB/5 (Item 5 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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09319325 97199048 PMID: 9047023
Immunogenicity and contraceptive potential of a human zona pellucida 3 peptide vaccine.

Bagavant H; Fusi FM; Baisch J; Kurth B; David CS; Tung KS
Department of Pathology, University of Virginia, Charlottesville 22908, USA. hb5u@uva.pcmail.virginia.edu

Biology of reproduction (UNITED STATES) Mar 1997, 56 (3) p764-70,
ISSN 0006-3363 Journal Code: A3W

Contract/Grant No.: AI 14764, AI, NIAID; P30 HD 28934, HD, NICHD; U54 HD 29909, HD, NICHD

Erratum in Biol Reprod 1997 May;56(5) 1361

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Immunization with zona pellucida 3 (ZP3) glycoprotein induces infertility in primates and is a target antigen for a contraceptive vaccine. However, loss of ovarian function is a long-term side effect. A possible mechanism is autoimmune ovarian disease induced by ZP3-specific autoreactive T cells, demonstrated in mice immunized with a murine ZP3 peptide in complete Freund's adjuvant. Indeed, a murine contraceptive vaccine that elicits antibodies to zona pellucida (ZP) without concomitant pathogenic T-cell activation has been achieved by a chimeric peptide (CP) consisting of a native ZP3 B-cell epitope and a foreign helper T-cell peptide. Herein, we evaluate the CP strategy in primate for human ZP3 (hZP3) vaccine development. A CP was constructed that consisted of a known helper T-cell epitope from the malarial circumsporozoite protein and a native B-cell epitope of hZP3. The human CP elicited antibodies to ZP3 in macaques without a measurable T-cell response to the hZP3 peptide. The serum antibodies reacted with macaque and human ZP and significantly inhibited human sperm binding to oocytes in vitro. Moreover, the CP elicited antibodies to human ZP in mice that lack murine major histocompatibility complex (MHC) class II molecules but express transgenic human HLA-DR3, -DQ6, or DQ8 molecules. Therefore, this study 1) provides evidence to support the feasibility of the CP strategy in hZP3 vaccine development and 2) describes a novel approach for evaluating the influence of polymorphic human MHC on vaccine immunogenicity without human immunization.

3/AB/6 (Item 6 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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09274715 97216236 PMID: 9062493

The role of relaxin in glycodelin secretion.

Stewart DR; Erikson MS; Erikson ME; Nakajima ST; Overstreet JW; Lasley BL; Amento EP; Seppala M

Department of Obstetrics and Gynecology, University of California, Davis 95616, USA.

Journal of clinical endocrinology and metabolism (UNITED STATES) Mar 1997, 82 (3) p839-46, ISSN 0021-972X Journal Code: HRB

Contract/Grant No.: P01ES06198, ES, NIEHS

Languages: ENGLISH

Document type: Clinical Trial; Journal Article

Record type: Completed

Glycodelin is a glycoprotein named for its unique carbohydrate structure. Glycodelin is produced by the secretory endometrium during the late luteal phase and returns to baseline during menses of the ensuing cycle, whereas in conceptive cycles it rapidly increases. Although progesterone and possibly estradiol are required for glycodelin production, they are not directly involved in the synthesis and release of this protein. Their role may be development of the endometrial secretory glandular elements, whereas other factors are required to initiate and maintain glycodelin secretion. The pattern of relaxin secretion during the luteal phase and early pregnancy is similar to that of glycodelin, but

their profiles have not been determined simultaneously. To investigate the relationship of relaxin and glycodelin, two studies were conducted. In the first study, relaxin, glycodelin, and ovarian steroids were measured in daily serum samples from nonconceptive and conceptive natural cycles. Profiles of relaxin and glycodelin were closely associated, with the onset of relaxin preceding glycodelin secretion by 1-2 days in nonconceptive cycles, and the pregnancy-associated increases in each hormone differing by about 2 days. The second study tested the hypothesis that relaxin stimulates glycodelin secretion. Samples were obtained from patients injected with human relaxin for 28 days. In subjects demonstrating ovarian cyclicity, glycodelin secretion was elevated, but it was not detected in subjects without ovarian cyclicity or in placebo-treated control subjects. This study reveals a close temporal and quantitative relationship between relaxin and glycodelin profiles in the late luteal phase and early pregnancy. It also demonstrates that relaxin administration can stimulate glycodelin production from a developed endometrium. This is the first report of a nonsteroidal ovarian factor that controls glycodelin secretion, and these results suggest a function for relaxin during early pregnancy. Glycodelin is a potent inhibitor of sperm zona pellucida binding by virtue of its extensive carbohydrate structure, but it is normally at a nadir in the periovulatory period. The data demonstrate that relaxin can stimulate glycodelin secretion throughout the menstrual cycle, including the periovulatory period, when relaxin-induced glycodelin secretion could have a contraceptive effect.

3/AB/7 (Item 7 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
(c) format only 2001 Dialog Corporation. All rts. reserv.

09272887 97173852 PMID: 9021751

Species-specific effect of oviductal glycoproteins on hamster sperm binding to hamster oocytes.

Schmidt A; Mavrogianis PA; O'Day-Bowman MB; Verhage HG

Department of Obstetrics and Gynecology, University of Illinois at Chicago 60612, USA.

Molecular reproduction and development (UNITED STATES) Feb 1997, 46

(2) p201-7, ISSN 1040-452X Journal Code: AN7

Contract/Grant No.: HD20571, HD, NICHD

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The secretory cells of the oviductal epithelium secrete a high-molecular-weight glycoprotein (OGP). OGPs from different mammalian species show similar immunological characteristics, their cDNAs show high homologies, and they associate with the zona pellucida of oviductal oocytes in vivo. The purpose of this study was to determine the effect of OGP obtained from different species on the binding of hamster sperm to hamster oocytes. Hamster oocytes were inseminated (30 min) in the presence or absence of homologous or heterologous OGPs, and sperm bound/oocyte were counted after removing loosely attached sperm. Ovarian oocytes had an average of 2.9 ± 0.6 sperm bound/oocyte, whereas oviductal oocytes had 36.3 ± 2.7 . Hamster OGP (0.1 mg/ml) significantly increased sperm binding to ovarian oocytes twofold and had no effect on sperm bound/oviductal oocytes. Human OGP (0.5 mg/ml) significantly decreased sperm binding to ovarian oocytes (0.9 ± 0.3 sperm bound/oocyte). This effect was dose dependent for oviductal oocytes and could be blocked

by preincubating human OGP with a specific antibody to human OGP. The presence of baboon and cow OGP during the insemination of hamster oviductal oocytes also resulted in a significant decrease in sperm bound/oocyte, whereas the addition of hamster OGP to hamster oviductal oocytes had no effect. These results show that homologous OGP enhances sperm binding to the ZP, whereas heterologous OGP inhibits that effect. Thus, our results suggest that OGP plays a role in the species-specific characteristics of sperm /ZP interaction, and that one must use a homologous system (OGP and gametes from the same species) to study the biological effect of OGP.

3/AB/8 (Item 8 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
(c) format only 2001 Dialog Corporation. All rts. reserv.

09227519 97105522 PMID: 8984181
Evaluation of zona pellucida antigens as potential candidates for immun contraception.

Paterson M; Wilson MR; van Duin M; Aitken RJ
MRC Reproductive Biology Unit, Centre for Reproductive Biology, Edinburgh, UK.

Journal of reproduction and fertility (ENGLAND) 1996, 50 p175-82,
ISSN 0449-3087 Journal Code: JWR

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Antibodies directed against the zona pellucida can interrupt sperm-egg recognition in vitro. However, the mechanisms by which anti-zona antibodies exert this contraceptive effect in vivo remain uncertain. There is an accumulating body of evidence to suggest that active immunity against zona antigens not only induces infertility via an antibody-mediated interruption of sperm-egg interaction but also disrupts normal ovarian function. We have evaluated the consequence of inducing active immunity against purified recombinant human ZP3 (rec.hZP3) and human ZP3 peptides, using the marmoset monkey, *Callithrix jacchus*, as an animal model. Although infertility was established in animals that received rec.hZP3, it was associated with ovarian dysfunction characterized by suppression of folliculogenesis and depletion of the primordial follicle pool. Immunization with continuous human ZP3 peptides, identified by epitope mapping studies, did not induce ovarian pathology but the antibody titres were insufficient to suppress fertility significantly and consistently. Clearly, further research is required to identify and segregate epitopes on the zona glycoproteins that are capable of inducing infertility without any side effects.

3/AB/9 (Item 9 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
(c) format only 2001 Dialog Corporation. All rts. reserv.

09169768 97185193 PMID: 9117283
Recombinant hamster sperm receptors that exhibit species-specific binding to sperm.

Litscher ES; Wassarman PM
Department of Cell Biology and Anatomy, Mount Sinai School of Medicine, New York, NY 10029, USA.

Zygote (ENGLAND) Aug 1996, 4 (3) p229-36, ISSN 0967-1994

Journal Code: B33

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Previous studies have shown that mouse sperm bind to hamster eggs and hamster sperm bind to mouse eggs in vitro. Furthermore, sperm receptor glycoprotein isolated from the zona pellucida of unfertilised hamster (hZP3) and mouse (mZP3) eggs binds to sperm from the heterologous species. Here, we expressed the hZP3 gene, under control of a constitutive promoter (pgk-1), in mouse embryonal carcinoma (EC) cells and Chinese hamster ovary (CHO) cells stably transfected with the hZP3 gene. In both cases, recombinant hZP3 (EC-hZP3 and CHO-hZP3) secreted into the culture medium was partially purified by high-performance liquid chromatography on a size-exclusion column and assayed for bioactivity using mouse and hamster gametes. Unlike hamster egg hZP3, which binds to both mouse and hamster sperm, EC-hZP3 and CHO-hZP3 exhibits species-specific binding to hamster sperm and induce hamster sperm, but not mouse sperm, to undergo the acrosome reaction in vitro. These results provide further evidence that species-specific binding of sperm to eggs in mammals is carbohydrate-mediated. Furthermore, the results suggest that recombinant forms of mammalian sperm receptors may be useful in assessing the molecular basis of species-specific fertilisation in mammals.

3/AB/10 (Item 10 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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09077727 97105520 PMID: 8984179

Influence of autoimmune ovarian disease pathogenesis on ZP3 contraceptive vaccine design.

Lou YH; Bagavant H; Ang J; McElveen MF; Thai H; Tung KS

Department of Pathology, University of Virginia, Charlottesville 22908, USA.

Journal of reproduction and fertility (ENGLAND) 1996, 50 p159-63, ISSN 0449-3087 Journal Code: JWR

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Zona pellucida (ZP) glycoproteins possess sperm receptor-binding activities. Antibodies against ZP can block sperm-egg interaction and thereby prevent fertilization. The feasibility of developing a safe contraceptive vaccine based on the ZP has been hampered by the finding that active immunization with autologous or heterologous ZP proteins results in infertility that is associated with ovarian dysfunction. A mouse model was used to investigate mechanisms of the ovarian pathology that is induced by active immunization with a 13mer peptide derived from mouse ZP3 (mZP3 (330-342)). This peptide includes one native B-cell epitope and two nested T-cell epitopes. Ovarian pathology could be transferred into naive recipients by CD4+ T cells, but not by antibodies, from immunized mice, suggesting the importance of T cells in the mechanism of ovarian pathogenesis. Moreover, immune responses, as well as disease induction, were restricted to H-2a,k,u,s,axb haplotypes. On the basis of this mouse model, a strategy to generate a contraceptive anti-ZP antibody response without a pathogenic T-cell response, irrespective of H-2 haplotype, is described. The B-cell epitope was modified by amino acid substitution to

eliminate the overlapping oophoritogenic T-cell epitope, and was linked to a promiscuous foreign T-cell epitope, bovine RNase94-104. The resultant chimaeric peptide (CP2) induced anti-ZP antibodies in 100% of the eight strains of inbred mice with different H-2 haplotypes without significant disease induction. An antifertility trial in B6AF1 female mice immunized with CP2 showed that the anti-ZP antibody was associated with a reduction in fertility. This infertility was reversed with a decline in anti-ZP antibody titre. Preliminary data show that this strategy of vaccine design may also be applied to primates.

3/AB/11 (Item 11 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
(c) format only 2001 Dialog Corporation. All rts. reserv.

09077725 97105518 PMID: 8984177

Molecular biology approaches to evaluate species variation in immunogenicity and antigenicity of zona pellucida proteins.

Prasad SV; Wilkins B; Dunbar BS

Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030, USA.

Journal of reproduction and fertility (ENGLAND) 1996, 50 p143-9,
ISSN 0449-3087 Journal Code: JWR

Contract/Grant No.: HD 17543, HD, NICHD

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Immun contraception using the glycoproteins of the mammalian zona pellucida (ZP) has held great promise because antibodies specific to ZP antigens would inhibit fertility and not be abortive. It has been shown, however, that some ZP proteins will elicit adverse effects since immunization may affect ovarian follicular development. These effects vary among different mammalian species as well as on the source of the ZP immunogen. Therefore, the use of molecular biology has been essential in identifying specific ZP protein(s) that inhibit fertility without altering ovarian follicular development and in defining the relationships of ZP proteins among different species. Use of recombinant ZP proteins has allowed us to begin to dissect antigenic domains of ZP proteins and to evaluate their potential roles in the fertilization process. Recent studies using recombinant rabbit ZP proteins to immunize cynomolgus monkeys (*Macaca fascicularis*) have shown that the 55 kDa ZP protein will elicit antibodies that inhibit sperm binding while not altering ovarian function, in contrast to immunization with a recombinant truncated protein of the 75 kDa ZP protein which causes ovarian dysgenesis. The rabbit 55 kDa protein is the homologue of the pig ZP3 alpha sperm receptor and the human ZPB protein but is distinct from the mouse ZP3 sperm receptor. Expression of this protein using the baculovirus expression system has further shown that the 55 kDa protein binds to capacitated rabbit spermatozoa over the acrosomal region and induces the acrosome reaction. Antibodies against this recombinant form of the ZP also inhibit rabbit spermatozoa from binding to rabbit egg in vitro. These studies demonstrate the need to determine the structure and function of ZP proteins of different mammalian species to evaluate their potential for contraceptive vaccines.

3/AB/12 (Item 12 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

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09044289 96404685 PMID: 8808826

[Elucidation of the mechanism of fertilization and clinical application of assisted reproductive technology]

Hiroi M

Department of Obstetrics and Gynecology, Yamagata University School of Medicine.

Nippon Sanka Fujinka Gakkai zasshi (JAPAN) Aug 1996; 48 (8) p578-90, ISSN 0300-9165 Journal Code: INR

Languages: JAPANESE

Document type: Journal Article; Review; Review, Tutorial

Record type: Completed

Fertilization is the process including many events such as maturation of egg and sperm, attachment, binding, acrosomal reaction, penetration, fusion, cortical reaction, zona reaction and nuclear fusion of both gamete, whereby individual gametes from the female and male unite to create offspring. Although the reason for mechanism of fertilization is still not clearly understood, this process may accelerate the rate adaptation in evolution. In this special lecture, I would like to present our experimental and clinical results especially concerning with morphological, physiological, biochemical and molecular approach on the mechanism of fertilization. 1. Development and maturation of follicles and oocytes. It is well known that pituitary FSH, LH control the ovarian function. Follicular development and ovum maturation are also controlled by both pituitary gonadotropins and local factors such as autocrine and paracrine agents. When hMG is injected during 1-6 day of menstrual cycle, several dominant follicles are developed. If hMG is injected after selection of dominant follicles, only one dominant follicle develop in the ovary. When PMS-treated immature rats were injected with immature or mature follicle fluids, rats injected with mature follicular fluid showed strongly suppress in the ovarian weights and numbers of ovulated follicles. Also mature follicle suppress aromatization from androstenedione to estradiol. These findings mean that mature follicular fluid contains inhibitory factors. Apoptosis of granulosa cells and follicular steroids are related to fertilization. 2. Intracellular calcium of oocyte. Intracellular calcium concentration is known to start to increase in a periodic manner after fertilization in oocytes of mammals. In 65% of tested mouse oocytes, fertilization occurred during 4 hours observation after sperm insemination in vitro. An initial long lasting intracellular calcium concentration was observed and followed by periodic manner. This calcium oscillation is inhibited by calcium blockers such as verpamil and nifedipine, but increased by high concentration of extracellular calcium concentration in the medium. Role of increase of intracellular calcium are understood to prevent polysperm and activate metabolism of oocytes. 3. Glucose metabolism of oocytes. Mouse embryo utilizes pyruvate as an essential nutrient until the 8-cell stage, and glucose thereafter. We have devised non-radiometric and enzymatic microassay method to measure glucose, deoxyglucose, deoxyglucose 6-phosphate incorporated into individual mouse oocytes and preimplantation embryo. In parallel, the activities of several enzymes of glycolytic pathway were also determined. In this study, glucose metabolism is necessary to develop in fertilized ova with changing activity of enzymes. 4. Molecular bases of ovarian fluid. The zona pellucida ZP is involved in a number of events in fertilization, all these fertilization events occur in the oviduct. Oviductal glycoprotein 200-240 KD has been identified from oviductal zona pellucida. Monoclonal antibody of oviductal glycoprotein reacted with ZP of oviductal egg but

not with the ovarian egg. Anti-ZPO antibody inhibit to bind sperm to ZP. Sequences in mouse and hamster oviduct specific glycoprotein are estimated, this glycoprotein mRNA was observed in only oviduct by northern blotting method. These molecular gene expression was observed by in situ hybridization in the oviduct of estrous cycle of hamster. 5. Microinsemination of sperm . Microinsemination of sperm into oocyte is widely used in clinical medicine. Sperm penetration assay (hamster test) is useful method to estimate fertilization capacity of sperm . But immotile sperm cannot estimate it. So modified micro sperm penetration assay was established to estimate fertilization capacity of sperm by using micro-manipulator. Subzonal sperm injection (SUZI) and intracytoplasmic sperm injection (ICSI) promotes fertilization and cleavage rate in immotile

3/AB/13 (Item 13 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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09026463 96435115 PMID: 8838001

Association of oviduct-specific glycoproteins with human and baboon (Papio anubis) ovarian oocytes and enhancement of human sperm binding to human hemizonae following in vitro incubation.

O'Day-Bowman MB; Mavrogianis PA; Reuter LM; Johnson DE; Fazleabas AT; Verhage HG

Department of Obstetrics and Gynecology, University of Illinois College of Medicine at Chicago 60612, USA.

Biology of reproduction (UNITED STATES) Jan 1996, 54 (1) p60-9,
ISSN 0006-3363 Journal Code: A3W

Contract/Grant No.: HD07579, HD, NICHD; HD20571, HD, NICHD

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The objectives of this study were 1) to determine whether or not human and baboon oviduct-specific glycoproteins (human OGP, baboon OGP) would associate with ovarian oocytes during in vitro incubation in a manner similar to that detected in vivo for oviductal oocytes and 2) to determine whether the association of OGP with ovarian oocytes influenced sperm binding. In vitro association of OGP with ovarian oocytes was assessed by indirect immunofluorescence assay using a polyclonal antibody prepared against human or baboon OGP. Human and baboon ovarian oocytes incubated in culture media containing OGP showed association of OGP with the zona pellucida (ZP) as detected by bright fluorescence. A similar pattern of fluorescence was observed in baboon oviductal oocytes (positive control). No fluorescence of the ZP was detected from ovarian oocytes incubated with culture medium alone. The pattern of fluorescence for ovarian oocytes incubated with OGP and serum albumin, the major oviductal fluid protein, was similar to that for oocytes incubated with OGP alone. A modified hemizona assay was used to assess whether association of human OGP with human ovarian oocytes influenced sperm binding. The number of sperm bound to hemizonae in the presence of human OGP was significantly greater ($p < 0.01$) than the number bound to hemizonae in the control culture medium. Addition of antibodies specific for human OGP to the incubation medium 1 h prior to addition of gametes blocked the enhancement of sperm binding seen in the presence of human OGP alone. Finally, human hemizona assays conducted in the presence of baboon OGP resulted in a significant decrease ($p < 0.05$) in the number of sperm bound per zona

compared with that in culture medium alone despite high homology between human and baboon OGP. These results 1) suggest that human OGP associates with ovulated oocytes in vivo; 2) support the hypothesis that association of OGP with the ZP may play a role in fertilization, possibly through enhancing the binding of sperm to the ZP within the oviduct; and 3) suggest that a homologous system (i.e., gametes and oviductal glycoprotein from the same species) is necessary for study of the function of oviductal glycoproteins .

3/AB/14 (Item 14 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
(c) format only 2001 Dialog Corporation. All rts. reserv.

09023375 96254637 PMID: 8962644
ZP3 peptide vaccine that induces antibody and reversible infertility without autoimmune oophoritis.
Tung KS; Ang J; Lou Y
Department of Pathology, University of Virginia, Charlottesville 22908, USA.

American journal of reproductive immunology (DENMARK) Mar 1996, 35
(3) p181-3, ISSN 1046-7408 Journal Code: AEZ
Contract/Grant No.: P30-HD 28934, HD, NICHD; U54HD29099, HD, NICHD
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Autoantibodies to ZP3 , a major glycoprotein of the zona pellucida (ZP) with sperm receptor function, can block sperm /oocyte interaction. However, only mice of certain major histocompatibility complex (MHC) haplotype respond to the ZP3 peptide. Moreover, ZP3 -specific T cells can mediate ovarian autoimmune disease. A chimeric peptide has been designed that induces antibody to native ZP3 regardless of the MHC haplotype of the inbred mice tested. This results in reduction in fertility that is reversible. Infertility correlates well with ZP antibody titer, and the mice do not develop concomitant autoimmune oophoritis. The vaccine contains (1) a promiscuous foreign T-cell peptide capable of eliciting a T-cell response regardless of the animals' MHC haplotype, and (2) a modified native B-cell peptide of ZP3 .

3/AB/15 (Item 15 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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09023374 96254636 PMID: 8962643
The potential of the zona pellucida as a target for immunocontraception.
Aitken RJ; Paterson M; van Duin M
MRC Reproductive Biology Unit, Edinburgh, Scotland.
American journal of reproductive immunology (DENMARK) Mar 1996, 35
(3) p175-80, ISSN 1046-7408 Journal Code: AEZ
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
PROBLEM: To investigate the contraceptive potential of the zona pellucida . METHOD: Generation of antibodies against native and recombinant zona glycoproteins which have then been assessed for their capacity to disrupt sperm -zona interaction in vivo and in vitro. The

animal model selected for these studies was the common marmoset and the end points examined were antibody titre, ovarian cyclicity and fertility. RESULTS: The fact that antibodies against the major zona glycoprotein , ZP3 , block both the primary and secondary phases of sperm -zona interaction suggests that this molecule might have potential for contraceptive vaccine development. Active immunization of marmoset monkeys with native porcine ZP3 or recombinant human ZP3 produced long term infertility but also precipitated a premature decline in the primordial follicle population. CONCLUSIONS: Future studies will have to determine whether a safe, effective vaccine can be engineered by coupling unique B-cell epitopes from ZP3 to foreign T-cell antigens.

3/AB/16 (Item 16 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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08895707 95274816 PMID: 7755176

Stage-specific immunolabeling for oviductin in the secretory granules of the oviductal epithelium of the golden hamster during the estrous cycle.

Roux E; Kan FW

Department of Anatomy, Faculty of Medicine, Universite de Montreal, Quebec, Canada.

Anatomical record (UNITED STATES) Mar 1995, 241 (3) p369-76, ISSN 0003-276X Journal Code: 4QM

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

BACKGROUND: We have previously localized an antigen of oviductal origin in the zona pellucida of postovulatory hamster ova. This antigen is a high molecular weight glycoprotein secreted by the non-ciliated secretory cells of the oviduct and is later transferred to the zona pellucida of the oocyte during oviductal transit. This glycoprotein is rich in N-acetyl-D-galactosamine residues and has been designated Hamster Oviductin-1. In the present study, a monoclonal antibody (MAb) raised against this oviductin was used to detect the presence of this antigen in oviductal tissue during the estrous cycle. METHODS: Twenty mature female golden hamsters were used and were divided into five groups of five animals each according to the five different stages of the estrous cycle. Quantitative immunocytochemistry was performed on MAb-labeled thin sections of Lowicryl-embedded ampullary region of hamster oviducts. Control experiments were also carried out to assess the specificity of the immunolabeling. RESULTS: Quantitative analysis of the immunogold labeling indicated that maximum labeling for oviductin in the secretory granules of oviductal epithelial secretory cells was found around the time of ovulation, i.e., at estrus. The intensity of immunolabeling decreased from metestrus to diestrus 1, was at a minimum at diestrus 2, and started to increase at proestrus. CONCLUSION: Together, these quantitative results indicate that expression of oviductin in the secretory granules of the hamster oviductal secretory cells is stage specific. Maximum labeling for the antigen coincides with the time of ovulation suggesting an important role for the oviductal epithelium in contributing its secretory product to the zona pellucida of oocytes freshly released from the ovary. Since the oviduct is the site of sperm -egg interaction and where fertilization and early embryo development take place, the maximal production of oviductin at the time of ovulation may facilitate some of these crucial steps during the intricate process of reproduction.

3/AB/17 (Item 17 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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08760617 96352277 PMID: 8750503

Response of cynomolgus macaques to immunization against a synthetic peptide from the human zona pellucida.

Mahi-Brown CA; Moran F
California Regional Primate Research Center, University of California, Davis, U.S.A.

Journal of medical primatology (DENMARK) Dec 1995, 24 (4) p258-70,
ISSN 0047-2565 Journal Code: J3Y

Contract/Grant No.: RR00169, RR, NCRR

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

This study tested immunogenicity of a synthetic peptide hZP3(327-341) from a human zona pellucida (ZP) glycoprotein. After antibody response to various peptide-carrier conjugates was assessed in mice, two female cynomolgus macaques were immunized with the peptide conjugated to keyhole limpet hemocyanin (KLH). A control macaque was immunized with KLH. The peptide was immunogenic in both species, and included both B and T cell epitopes since low to moderate titers of peptide-specific antibodies and a T cell proliferative response were measured. Profiles of ovarian steroid metabolites indicated unchanged ovarian function in the macaques, but only the control conceived when bred. Ovarian histology was normal except that immunoglobulin was bound to ZP in follicles of the peptide-immune macaques. ZP from these females bound sperm and induced acrosome reactions at rates equal to those of an untreated control. The results support the feasibility of an immunocontraceptive vaccine based on autologous ZP peptides.

3/AB/18 (Item 18 from file: 155)
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08601952 95392042 PMID: 7663023

Transgenic mice with reduced numbers of functional sperm receptors on their eggs reproduce normally.

Liu C; Litscher ES; Wassarman PM
Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110-1199, USA.

Molecular biology of the cell (UNITED STATES) May 1995, 6 (5)
p577-85, ISSN 1059-1524 Journal Code: BAU

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

To initiate fertilization in mice, free-swimming sperm bind to mZP3, an approximately 83-kDa glycoprotein present in the ovulated egg zona pellucida (ZP). mZP3 is located periodically along the filaments that constitute the ZP. Sperm recognize and bind to specific oligosaccharides linked to one or more of five Ser residues clustered in the carboxy-terminal one-third of the mZP3 polypeptide. When all five Ser residues are converted to nonhydroxy amino acids by site-directed

mutagenesis of the mZP3 gene, an inactive form of mZP3, called mZP3 [ser], is secreted by embryonal carcinoma cells stably transfected with the mutated gene. Here, seven independent transgenic mouse lines were established that harbor the mutated mZP3 gene. In all lines, the mutant gene is expressed by growing oocytes and mZP3 [ser] is synthesized, secreted, and incorporated into the ZP. Purified mZP3 [ser] prepared from ovaries of transgenic mice, like mZP3 [ser] from transfected embryonal carcinoma cells, is inactive in sperm binding assays in vitro. On the other hand, the presence of mZP3 [ser] in the ZP does not significantly affect either the binding of sperm to ovulated eggs in vitro or the reproduction of the mice, i.e., the transgenic mice are fertile, breed at normal intervals, and produce litters of normal sizes. These results indicate that the number of functional sperm receptors in the ZP can be reduced by more than 50% without adversely affecting fertilization of eggs in vivo.

3/AB/19 (Item 19 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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08592628 95378696 PMID: 7650399

A zona pellucida 3 peptide vaccine induces antibodies and reversible infertility without ovarian pathology.

Lou Y; Ang J; Thai H; McElveen F; Tung KS
Department of Pathology, University of Virginia, Charlottesville 22908, USA.

Journal of immunology (UNITED STATES) Sep 1 1995, 155 (5) p2715-20,
ISSN 0022-1767 Journal Code: IFB

Contract/Grant No.: HD 28934, HD, NICHD; HD 29099, HD, NICHD

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Zona pellucida 3 (ZP3) is a major glycoprotein of the zona pellucida that possesses the sperm receptor function. ZP3 induces autoantibody that can block sperm/oocyte interaction. However, the feasibility of a ZP3 contraceptive vaccine has been marred by the finding that ZP3-specific T cells mediate ovarian autoimmune disease. Moreover, as reported in this work, only some inbred mouse strains respond to the ZP3 peptide. We now describe a chimeric peptide that induces Abs to native ZP3 regardless of the MHC haplotype of the inbred mice tested. Study in one mouse strain resulted in reduction in fertility that correlates well with zona pellucida Ab titer, and most importantly, the mice do not develop concomitant autoimmune oophoritis. Moreover, the infertility was completely reversible. The design of the vaccine chimeric peptide is governed by the inclusion of two essential components: 1) a promiscuous foreign T cell peptide capable of eliciting a Th cell response regardless of the MHC haplotype of the animals, and 2) the native B cell peptide of ZP3 that has been modified by substitution of residue(s) critical for T cell but not B cell response to ZP3.

3/AB/20 (Item 20 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
(c) format only 2001 Dialog Corporation. All rts. reserv.

08565582 95349014 PMID: 7623323

Oviduct proteins in fertilization and early embryo development.

Nancarrow CD; Hill JL

CSIRO Division of Animal Production, NSW, Blacktown, Australia.

Journal of reproduction and fertility (ENGLAND) 1995, 49 p3-13,

ISSN 0449-3087 Journal Code: JWR

Languages: ENGLISH

Document type: Journal Article; Review; Review, Academic

Record type: Completed

The oviduct controls the environment in which the gametes are transported and fuse, and in which embryonic development begins. The ultrastructural topography of the ampulla and isthmus is similar, consisting of ciliated and secretory cells, but a different array of proteins is secreted by each segment along with various serum components. Amino acids are selectively secreted by the oviduct; these amino acids probably interact with the gametes or embryo to facilitate the processes of fertilization and development. An oviduct-specific glycoprotein is synthesized by the ampulla of sheep and cattle in response to oestrogen and secreted mainly from day-1 to day 3 of the ovarian cycle. This oestrus-associated glycoprotein (EGP) has a variable molecular mass of 80-97 kDa and a pI value ranging from 4.7 to 5.5. The bovine (b) and ovine (o) EGP genes are 95.5% identical and consist of 1560 base pairs encoding 519 amino acids containing one N-linked and several O-linked glycosylation sites. The terminal glycosides are N-acetylglucosamine and galactose-N-acetylgalactosamine for bEGP, and fucose, galactose and sialic acid residues are also identified for oEGP. EGP binds to zona pellucida and blastomere membranes, but evidence for EGP binding to sperm membranes is equivocal. After in vitro fertilization the proportion of sheep oocytes cleaving was increased in the presence of oEGP, but when single-cell embryos were cultured with oEGP, these cleavage rates were reduced. In addition, consistent positive effects of oEGP were observed on blastocyst formation. Elaboration of the mechanism of synthesis of EGP, its action and its role in fertilization and embryo development is important for our understanding of the events of early pregnancy.

3/AB/21 (Item 21 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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08233023 94361823 PMID: 8080645

In vitro incubation of golden (Syrian) hamster ovarian oocytes and human sperm with a human oviduct specific glycoprotein.

Reuter LM; O'Day-Bowman MB; Mavrogianis PA; Fazleabas AT; Verhage HG

Department of Obstetrics and Gynecology, University of Illinois College of Medicine, Chicago 60612-7313.

Molecular reproduction and development (UNITED STATES) Jun 1994, 38

(2) p160-9, ISSN 1040-452X Journal Code: AN7

Contract/Grant No.: HD20571, HD, NICHD

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The objective of this study was to determine if human oviduct specific glycoprotein (huOGP) would associate with hamster ovarian oocytes and human sperm during in vitro incubation. The huOGP used in these studies was partially purified from human hydrosalpinx fluid. Hamster ovarian oocytes and human sperm samples were incubated in culture medium with and without huOGP. Association of huOGP was assessed by indirect immunofluorescence

assay using a polyclonal antibody prepared against huOGP. Intense fluorescence of the zona pellucida, and bright but uneven fluorescence of the perivitelline space, were observed in hamster ovarian oocytes following incubation in the presence of huOGP. A similar but more uniform pattern of fluorescence was observed when hamster oviductal oocytes (positive controls) were incubated in culture medium alone. Fluorescence was absent when oocytes were assayed with preimmune serum. The association of huOGP with the zona pellucida and perivitelline space appeared to be specific since thyroglobulin, a large molecular weight glycoprotein, and human serum albumin, the major protein in oviduct fluid, did not associate with the hamster oocytes nor inhibit huOGP association when included in the culture medium. Fluorescence was absent when human sperm incubated with huOGP were assayed with antiserum to huOGP. However, human sperm fluoresced when incubated with a uterine glycoprotein, CUPED, which had previously been shown to bind to cat sperm during in vitro incubation. Sperm also fluoresced brightly when human sperm antibody was used as a positive control. Solubilization of sperm membrane proteins postincubation and analysis of these proteins by 1-D SDS-PAGE followed by immunoblotting also failed to show an association of huOGP with human sperm. Electron microscopy of sperm both pre- and postsolubilization confirmed that the sperm membranes were removed by this process. In conclusion, the association of huOGP with hamster oocytes in vitro suggests that huOGP may associate with human oocytes in vivo, whereas that may not be true for human sperm in vivo. The association of huOGP with oocytes may serve to facilitate the process of fertilization and early embryonic development within the oviduct.

3/AB/22 (Item 22 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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08214599 94337987 PMID: 8059976

Cytochemical characterization of oligosaccharide side chains of the glycoproteins of rat zona pellucida: an ultrastructural study.

Aviles M; Martinez-Menarguez JA; Castells MT; Madrid JF; Ballesta J
Department of Cell Biology, School of Medicine, University of Murcia, Spain.

Anatomical record (UNITED STATES) Jun 1994, 239 (2) p137-49, ISSN 0003-276X Journal Code: 4QM

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

BACKGROUND: The zona pellucida (ZP), an extracellular matrix which surrounds mammalian oocytes, is formed by different glycoproteins. Several studies have revealed that carbohydrate residues present in glycoproteins of ZP play a key role in the sperm-egg recognition. However, the origin and the biochemical composition of ZP remain to be completely resolved. METHODS: ZP glycoproteins from rat ovarian follicles were investigated at light and electron microscopic level by the application of lectins conjugated to peroxidase, digoxigenin, and colloidal gold in combination with enzyme and chemical treatment. A quantitative analysis was also performed. RESULTS: ZP shows reactivity to WGA, DSA, LFA, AAA, RCA I, and MAA. SBA and PNA showed a variable reactivity ranging from negative to strongly positive. A uniform pattern of binding throughout ZP was observed with DSA, Con A, AAA, MAA, and LFA. However, labeling by RCA I and SBA was higher in the outer ZP while PNA and WGA showed a higher

binding in the inner ZP. Lectin reactivity was detected in cortical granules, endoplasmic reticulum, Golgi apparatus, vesicles, and multivesicular bodies of oocytes. CONCLUSIONS: ZP contained the terminal disaccharides Gal beta 1,4GlcNAc, Gal beta 1,3GalNAc, and GalNAc beta 1,3Gal and the trisaccharides Neu5Ac alpha 2, 3Gal beta 1,4GlcNAc, Neu5Ac-Gal beta 1,3GalNAc, and Neu5Ac-GalNAc beta 1,3Gal sequences. The occurrence of Fucose residues alpha 1,6 linked to the inner core region of N-linked glycoproteins of ZP was demonstrated by the use of several fucose-specific lectins. Methylation-saponification treatment in combination with lectin cytochemistry reveals that Gal, GalNAc, and polyllactosamine residues of rat ZP glycoproteins contain sulphated groups. The reactivity observed in ooplasmic vesicles was similar to that of ZP, thus suggesting that the oocyte is the site of synthesis of ZP glycoproteins .

3/AB/23 (Item 23 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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08019573 94363314 PMID: 8081814

Cloning, sequencing and oocyte-specific expression of the marmoset sperm receptor protein, ZP3.

Thillai-Koothan P; van Duin M; Aitken RJ

MRC Reproductive Biology Unit, Edinburgh, UK.

Zygote (ENGLAND) May 1993, 1 (2) p93-101, ISSN 0967-1994

Journal Code: B33

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The zona pellucida surrounding the mammalian oocyte contains a major glycoprotein species, ZP3 , that serves as a cell- and species-specific receptor for spermatozoa . In this study we have determined the primary amino acid structure of marmoset ZP3 (marZP3) and examined the expression of marZP3 mRNA within the ovary . The marZP3 gene possesses an open reading frame of 1272 nucleotides which is expressed specifically by the oocyte and encodes a polypeptide chain of 424 amino acids that exhibits 91% homology with the human ZP3 sequence. The disparity between these molecules was confined to a short domain spanning residues 322-352; otherwise the molecules were very similar, showing conservation of many structural features including the N-linked glycosylation sites, location and number of cysteine and proline residues and hydrophobicity profile. The results of this study have important implications for the use of the marmoset monkey as an animal model for the development of contraceptive vaccines targeting ZP3 .

3/AB/24 (Item 24 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07950934 94046763 PMID: 8229931

Molecular biological methods for monitoring oocyte maturation and in vitro fertilization in bitches.

Nickson DA; Boyd JS; Eckersall PD; Ferguson JM; Harvey MJ; Renton JP

Department of Surgery/Reproduction, University of Glasgow Veterinary School, UK.

Journal of reproduction and fertility (ENGLAND) 1993, 47 p231-40,
ISSN 0449-3087 Journal Code: JWR
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

Oocytes were collected from ovaries of bitches, at various stages of the oestrous cycle, after routine sterilization. Cumulus-enclosed oocytes were cultured for 0-72 h in a modified M-199 medium containing 10% oestrous bitch serum and 20 micrograms oestradiol ml⁻¹. For oocytes surrounded by two or more layers of cumulus cells, an increase in the expression of mRNA transcripts for zona pellucida glycoprotein 3 (ZP3) was seen and reached peak levels after 48 h culture in vitro. Histological assessment showed that 39% of these oocytes had extruded their first polar body after 24 h culture in vitro. When these in vitro matured oocytes were transferred to oviduct cell monolayers and inseminated with fresh dog spermatozoa in Medium-199 supplemented with 10% fetal calf serum and 50 mg gentamicin sulfate ml⁻¹, penetration of the zona pellucida started 1 h after insemination for oocytes that had been cultured for 48 and 72 h. At 12 h after insemination both male and female pronuclei were seen in 37.5% and 20% of the oocytes incubated for 48 and 72 h, respectively. No further development was seen.

3/AB/25 (Item 25 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07845923 92239155 PMID: 1571163

Activation of a G protein in mouse sperm by the zona pellucida, an egg-associated extracellular matrix.

Wilde MW; Ward CR; Kopf GS

Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia.

Molecular reproduction and development (UNITED STATES) Apr 1992, 31

(4) p297-306, ISSN 1040-452X Journal Code: AN7

Contract/Grant No.: HD 06274, HD, NICHD; HD 07225, HD, NICHD; T32HD 07305, HD, NICHD; +

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Mammalian sperm possess a guanine nucleotide-binding regulatory protein (G protein), with properties similar to Gi, that appears to be involved in the signal transduction pathway required for zona pellucida (ZP)-mediated acrosomal exocytosis. Mouse sperm treated with pertussis toxin (PT), a toxin that functionally inactivates Gi proteins, bind to the ZP of mouse eggs but are inhibited from undergoing acrosomal exocytosis. We have measured high-affinity GTPase activity and GTP gamma [35S] binding in mouse sperm homogenates incubated in the absence and presence of ZP glycoproteins isolated from either ovulated eggs or from ovarian homogenates to determine whether this extracellular matrix can activate the sperm-associated Gi protein. An increase in GTP hydrolysis (approximately 50% over basal activity) and GTP gamma [35S] binding (approximately 25-60% over basal activity) is observed when sperm homogenates are incubated in the presence of solubilized ZP glycoproteins, and the increase in GTPase activity is dependent on the concentration of ZP added to the homogenates. Accompanying this increase is a reduction in the ability of PT to catalyze in vitro [32P]ADP-ribosylation of a Mr = 41,000 sperm Gi protein,

suggesting that the increase in GTPase activity and GTP gamma [35S] binding is associated with the activation of a PT-sensitive sperm G protein(s). The ability of the ZP to stimulate high-affinity GTPase activity in these homogenates appears to be dependent on the capacitation state of the sperm from which the homogenates are prepared. These data suggest that a component(s) of the ZP may function in a manner similar to that of other ligands by binding to a sperm surface-associated receptor and subsequently activating a G protein coupled to an intracellular signal transduction cascade(s) required for induction of acrosomal exocytosis.

3/AB/26 (Item 26 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07698332 93186207 PMID: 1293021

Characteristics of monoclonal antibodies against porcine zona pellucida-3 and their functional relevance.

Gupta SK; Bagavant H; Koothan PT; Talwar GP; Yurewicz EC; Sacco AG
National Institute of Immunology, Shahid Jeet Singh Marg, New Delhi.
Indian journal of experimental biology (INDIA) Nov 1992, 30 (11)
p1000-5, ISSN 0019-5189 Journal Code: GIZ
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

Seven monoclonal antibodies (MAs) against 55 kDa glycoprotein family of porcine zona pellucida (ZP3) reacting with either ZP3 alpha (MA-7, MA-27, MA-28) or ZP3 beta (MA-1, MA-2, MA-11, MA-30) have been described. MA-1, -2, -27, -28 and -30 do not recognize carbohydrate determinants as shown by their reactivity to the deglycosylated (DG) ZP3 alpha and ZP3 beta. Indirect immunoperoxidase studies showed that all MAs reacted with zona pellucida from porcine and monkey ovaries. Only MA-1 and -27 reacted with ZP from rabbit ovary as well, while none of the MAs recognised mouse ZP, MA-7, -11, -27, -28 and -30 inhibited in vitro, the zona lysis by trypsin as well as the binding of ZP3 to sperm membrane vesicle as investigated by ELISA.

3/AB/27 (Item 27 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07469318 92074587 PMID: 1741484

The hemizona assay (HZA) as an experimental model to evaluate the inhibition of sperm binding to the murine zona pellucida by isolated zona pellucida protein.

Windt ML; Franken DR; de Beer PM; Bouic PJ; Kruger TF
Department of Obstetrics and Gynaecology, University of Stellenbosch, Republic of South Africa.
Andrologia (GERMANY) May-Jun 1991, 23 (3) p209-12, ISSN 0303-4569
Journal Code: 4QP

Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Compelling evidence has demonstrated that zona binding represents gamete recognition by sperm binding with high affinity and specificity to complex glycoproteins of the zona pellucida. In the present study we evaluated

the hemizona assay (HZA) in the investigation of the interaction of mouse spermatozoa with unfertilized murine oocytes and hemizonae after exposure to solubilized murine zonae pellucidae proteins. The zonae pellucidae were isolated from ovarian tissue following described mincing techniques. The sperm binding characteristics of murine spermatozoa were studied by using SDS-PAGE isolated ZP2 (+/- 120 Kd) and ZP3 (+/- 83 Kd) components of the zona pellucida. Sperm receptor activity was examined in a competitive gamete binding fashion using the HZA as an indicator of sperm/zona interaction. The results illustrated that isolated, solubilized ZP2 and ZP3 glycoprotein moieties of the zona pellucida inhibited sperm binding to hemizonae and oocytes when compared to controls, and that the HZA can be utilized as an internally controlled homologous bioassay to evaluate the effects of zona pellucida proteins on tight binding of spermatozoa to the zona pellucida.

3/AB/28 (Item 28 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07342374 90152139 PMID: 2154392

Structural and functional relationships between mouse and hamster zona pellucida glycoproteins.

Moller CC; Bleil JD; Kinloch RA; Wassarman PM
Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110.

Developmental biology (UNITED STATES) Feb 1990, 137 (2) p276-86,
ISSN 0012-1606 Journal Code: E7T

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The hamster egg's extracellular coat, or zona pellucida, consists of three glycoproteins, designated hZP1, hZP2, and hZP3, that exhibit extensive heterogeneity on SDS-PAGE. hZP1 is a relatively minor component of hamster zonae pellucidae, as compared with hZP2 and hZP3. In the presence of reducing agents, hZP1, 200,000 apparent Mr, migrates on SDS-PAGE with an apparent Mr of 103,000. This suggests that hZP1, like mouse ZP1, is composed of two polypeptides held together by intermolecular disulfides. When purified hamster ZP glycoproteins were tested at relatively low concentrations in an in vitro competition assay, employing either hamster or mouse gametes, only hZP3 (56,000 apparent Mr) exhibited sperm receptor activity (i.e., inhibited binding of sperm to eggs). Thus, apparently hZP3 is the hamster counterpart of mouse ZP3, the mouse egg receptor for sperm. Furthermore, at relatively high concentrations, solubilized hamster egg ZP preparations induced both hamster and mouse sperm to undergo the acrosome reaction in vitro. hZP3 is encoded by a relatively abundant ovarian mRNA that is detected by a mouse ZP3 cDNA probe and is the same size, about 1.5 kb, as mRNA encoding the mouse sperm receptor, ZP3 (83,000 apparent Mr). Like mouse ZP2, hZP2 undergoes limited proteolysis following artificial activation of hamster eggs in vitro. Results of in vitro assays employing intact eggs and isolated zonae pellucidae demonstrate that hamster eggs possess a ZP2-proteinase which has a substrate specificity similar to that of the mouse enzyme. These observations are discussed in terms of structural and functional relationships that may exist between hamster and mouse zona pellucida glycoproteins.

3/AB/29 (Item 29 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07278044 90384978 PMID: 2402504

An upstream region of the mouse ZP3 gene directs expression of firefly luciferase specifically to growing oocytes in transgenic mice.

Lira SA; Kinloch RA; Mortillo S; Wassarman PM

Department of Cell and Developmental Biology, Roche Research Center, Nutley, NJ 07110.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Sep 1990, 87 (18) p7215-9, ISSN 0027-8424
Journal Code: PV3

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The gene encoding the mouse egg primary receptor for sperm, a zona pellucida glycoprotein called ZP3, is expressed exclusively in growing oocytes within ovaries of sexually immature and mature female mice. We have constructed a transgene in which 6.5 kilobases of ZP3 gene 5'-flanking sequence is fused to the coding region of the firefly luciferase gene, and we have generated four independent lines of transgenic mice. In these animals, the transgene is expressed exclusively in ovaries. Furthermore, within ovaries, expression is confined to growing oocytes, and luciferase activity can be detected by assaying individual, isolated oocytes. The pattern of firefly luciferase expression during oocyte growth is similar to that observed in previous studies of ZP3 expression during oogenesis in mice. Observations reported here strongly suggest that cis-acting elements present in the ZP3 gene 5'-flanking region regulate oocyte-specific and, therefore, sex-specific expression of the sperm receptor gene during mouse development. They also suggest that such elements can be used to direct expression of cloned genes specifically to oocytes of transgenic mice and to evaluate the effects of such expression on various aspects of early mammalian development.

3/AB/30 (Item 30 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
(c) format only 2001 Dialog Corporation. All rts. reserv.

07239362 90349545 PMID: 2385582

Human homolog of the mouse sperm receptor.

Chamberlin ME; Dean J

Laboratory of Cellular and Developmental Biology, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Aug 1990, 87 (16) p6014-8, ISSN 0027-8424
Journal Code: PV3

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The human zona pellucida, composed of three glycoproteins (ZP1, ZP2, and ZP3), forms an extracellular matrix that surrounds ovulated eggs and mediates species-specific fertilization. The genes that code for at least two of the zona proteins (ZP2 and ZP3) cross-hybridize with other

mammalian DNA. The recently characterized mouse sperm receptor gene (Zp - 3) was used to isolate its human homolog. The human homolog spans approximately 18.3 kilobase pairs (kbp) (compared to 8.6 kbp for the mouse gene) and contains eight exons, the sizes of which are strictly conserved between the two species. Four short (8-15 bp) sequences within the first 250 bp of the 5' flanking region in the human Zp -3 homolog are also present upstream of mouse Zp - 3 . These elements may modulate oocyte-specific gene expression. By using the polymerase chain reaction, a full-length cDNA of human ZP3 was isolated from human ovarian poly(A)+ RNA and used to deduce the structure of human ZP3 mRNA. Certain features of the human and mouse ZP3 transcripts are conserved. Both have unusually short 5' and 3' untranslated regions, both contain a single open reading frame that is 74% identical, and both code for 424 amino acid polypeptides that are 67% the same. The similarity between the two proteins may define domains that are important in maintaining the structural integrity of the zona pellucida , while the differences may play a role in mediating the species-specific events of mammalian fertilization.

3/AB/31 (Item 31 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07152662 94145584 PMID: 7508719

Mapping of immunogenic domains on porcine zona pellucida 3 alpha and beta glycoproteins by murine monoclonal antibodies.

Gupta SK; Bagavant H; Chadha K; Gupta M; Yurewicz EC; Sacco AG
Gamete Antigen Laboratory, National Institute of Immunology, New Delhi, India.

American journal of reproductive immunology (DENMARK) Sep-Oct 1993, 30
(2-3) p95-100, ISSN 1046-7408 Journal Code: AEZ

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

PROBLEM: Immunization with zona pellucida (ZP) leads to block in fertility with variable degree of ovarian dysfunctions. To design an immunocontraceptive vaccine based on synthetic peptides of zona pellucida, it is imperative to identify and define epitopes involved in sperm binding. METHOD: Epitopic domains recognized by monoclonal antibodies (MAbs) specific to either porcine ZP3 alpha or ZP3 beta glycoproteins were delineated in an enzyme-linked immunosorbent assay (ELISA) based on the ability of a MAb in solution to inhibit the binding of biotinylated ZP3 to another MAb coated on a microtitration plate. Immunoblot studies were carried out to determine the nature of reactive determinants. Porcine oocytes preincubated with MAbs were tested for sperm binding in vitro. RESULTS: Out of 23 MAbs generated, 10 had specificity for ZP3 alpha and 13 for ZP3 beta. By using these antibodies, eight epitopic domains on both ZP3 alpha and ZP3 beta were discernible. On ZP3 beta, epitopic domain DI partially overlaps with DII and DV with DVI, whereas on ZP3 alpha domains DI to DV were in close proximity with a partial overlap, suggesting the dominance of this region. All 10 MAbs against ZP3 alpha, and 10 out of 13 against ZP3 beta recognized deglycosylated forms of antigens. Seven antibodies having specificities for ZP3 alpha and ZP3 beta respectively recognized linear epitopes. MA-30, having specificity for ZP3 beta and MA-420 for ZP3 alpha and recognizing linear epitopes significantly inhibit the binding of boar sperm to porcine oocytes in vitro. CONCLUSIONS: Collectively, these studies indicate the value of

utilizing MAbs for identifying and characterizing functionally significant ZP determinants. MAbs recognizing sequential epitopes will help in the elucidation of the amino acid sequence of the epitopes, which will subsequently help in design of synthetic immunocontraceptive vaccines.

3/AB/32 (Item 32 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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06605445 88242926 PMID: 3378665

Molecular analysis of cDNA coding for ZP3, a sperm binding protein of the mouse zona pellucida.

Ringuette MJ; Chamberlin ME; Baur AW; Sobieski DA; Dean J
Laboratory of Cellular and Developmental Biology, National Institutes of Health, Bethesda, Maryland 20892.

Developmental biology (UNITED STATES) Jun 1988, 127 (2) p287-95,
ISSN 0012-1606 Journal Code: E7T

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

At fertilization, mammalian sperm bind in a species-specific manner to the extracellular zona pellucida that surrounds ovulated eggs. ZP3, an 83,000-85,000 Da glycoprotein of the murine zona pellucida, has been shown to inhibit sperm binding via its O-linked oligosaccharide side chains. We have recently isolated cDNA clones coding for ZP3 and have demonstrated that ZP3 transcripts are accumulated in oocytes where their expression is developmentally regulated during oogenesis. We now report that ZP3 mRNA is 1317 nt long with an estimated poly(A) tail of 200-300 nt. The short 29-nt 5' untranslated region is followed by a single open reading frame coding for a polypeptide chain of 46,307 Da which includes six possible sites for N-linked oligosaccharides. The N-terminus of ZP3 contains a potential 22-amino acid signal peptide which upon cleavage would result in a secreted core protein of 43,943 Da. The termination codon is a part of the AATAAA polyadenylation signal and is contained in an unusually short 16-nt 3' untranslated region. Sequences homologous to ZP3 are conserved among mammals and are expressed in ovarian tissue as mature transcripts with indistinguishable molecular weights.

3/AB/33 (Item 33 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
(c) format only 2001 Dialog Corporation. All rts. reserv.

05935726 89214643 PMID: 2496140

Differences between mature ovarian and oviductal oocytes: a study using the golden hamster.

Yang CH; Yanagimachi R
Department of Anatomy and Reproductive Biology, Hawaii School of Medicine 96822.

Human reproduction (ENGLAND) Jan 1989, 4 (1) p63-71, ISSN 0268-1161
Journal Code: HRP

Contract/Grant No.: HD-03402, HD, NICHD

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

To investigate whether mature ovarian oocytes are physiologically

identical with recently ovulated oocytes, hamster oocytes collected from ovaries approximately 1 h before ovulation were compared with the oocytes which had been in the oviduct for 3-4 h. Although these two groups of oocytes were at the metaphase of the second meiosis, there were quantitative differences between the two with respect to (i) sensitivity of cumulus matrix to hyaluronidase and spermatozoa (oviductal greater than ovarian), (ii) size of the perivitelline space (oviductal greater than ovarian), (iii) viscosity of the ooplasm (ovarian greater than oviductal), (iv) responsiveness to Ca^{2+} -ionophore (oviductal greater than ovarian), and (v) time needed in completing meiosis (ovarian greater than oviductal). The most prominent difference was found in the zona pellucida . The zona of the oviductal oocyte was 'heterogeneous' in its optical density and had a stronger acrosome-reaction-inducing ability than that of the ovarian oocyte. When cultured in artificial media (e.g. F-10 medium with serum) for 4 h, the ooplasm of ovarian oocytes became like that of oviductal oocytes. However, their zonae remained unchanged. Zonae of ovarian oocytes became like those of oviductal oocytes only when they were exposed to ampullary and/or isthmic fluids. The zona-altering factors in the oviductal fluid (oviductal glycoproteins), which are apparently integrated into the native zona, may act to enhance the various functions of the zona.

3/AB/34 (Item 34 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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05580480 90049231 PMID: 2479101

Vaccination with a synthetic zona pellucida peptide produces long-term contraception in female mice.

Millar SE; Chamow SM; Baur AW; Oliver C; Robey F; Dean J
Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD 20892.

Science (UNITED STATES) Nov 17 1989, 246 (4932) p935-8, ISSN 0036-8075 Journal Code: UJ7

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The zona pellucida surrounding mouse oocytes is an extracellular matrix composed of three sulfated glycoproteins , ZP1, ZP2, and ZP3 . It has been demonstrated that a monoclonal antibody to ZP3 injected into female mice inhibits fertilization by binding to the zona pellucida and blocking sperm penetration. A complementary DNA encoding ZP3 was randomly cleaved and 200- to 1000-base pair fragments were cloned into the expression vector lambda gt11. This epitope library was screened with the aforementioned contraceptive antibody, and the positive clones were used to map the seven-amino acid epitope recognized by the antibody. Female mice were immunized with a synthetic peptide containing this B cell epitope coupled to a carrier protein to provide helper T cell epitopes. The resultant circulating antibodies to ZP3 bound to the zona pellucida of immunized animals and produced long-lasting contraception. The lack of ovarian histopathology or cellular cytotoxicity among the immunized animals may be because of the absence of zona pellucida T cell epitopes in this vaccine.

3/AB/35 (Item 35 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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05054766 87152578 PMID: 3825684

Effects of anti-zona pellucida monoclonal antibodies on fertilization and early development.

Dean J; East IJ

Advances in experimental medicine and biology (UNITED STATES) 1986, 207 p37-53, ISSN 0065-2598 Journal Code: 2LU

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The murine zona pellucida surrounds the growing oocyte, ovulated egg and dividing embryo. It is comprised of three sulfated glycoproteins designated ZP-1, ZP-2, and ZP - 3 which have molecular weights of 185,000, 140,000 and 83,000 daltons respectively. We have isolated a series of cell lines that produce monoclonal antibodies to ZP-2 and to ZP - 3 . These immunological probes indicate that the extracellular matrix proteins of the zona pellucida are found uniquely in the ovary where they surround maturing oocytes. We have demonstrated that passive immunization with antibodies specific either for ZP-2 or ZP - 3 inhibit in vivo and in vitro fertilization. This effect is observed with ng/ml quantities of antibody. It appears that the antibodies do not preclude sperm binding but rather prevent sperm penetration of the zona by steric hinderance. Although long-term, the contraceptive effect is fully reversible and this reversibility is associated with loss of antibody from the zona pellucida surrounding intra-ovarian oocytes. Antibodies to ZP-2 or ZP - 3 had no other adverse effect on in vivo or in vitro preimplantation development.

3/AB/36 (Item 36 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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04659954 84237038 PMID: 6376213

Monoclonal antibodies to the major protein of the murine zona pellucida: effects on fertilization and early development.

East IJ; Mattison DR; Dean J

Developmental biology (UNITED STATES) Jul 1984, 104 (1) p49-56, ISSN 0012-1606 Journal Code: E7T

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The growing murine oocyte is surrounded by an extracellular zona pellucida consisting of three sulfated glycoproteins , ZP-1, ZP-2, and ZP - 3 . The smallest of these, ZP - 3 , has been reported to be the species-specific sperm receptor. Monoclonal antibodies have been recently characterized to three different antigenic determinants, two found exclusively on ZP-2, and one found on both ZP-2 and ZP - 3 . The in vivo effect of these antibodies on the three known functions of the zona pellucida were examined. The most dramatic effect was the prevention of fertilization. After administration, the monoclonal antibodies were located in the ovary on the zona pellucida of growing oocytes. Eggs ovulated subsequently were coated with the monoclonal antibodies and failed to develop into 2-cell embryos after mating. Eighty days later, the monoclonal antibodies could no longer be detected on the zona of ovarian oocytes,

and this loss coincided with the resumption of fertility. These findings provide molecular evidence for the hypothesis that the immunological block to sperm-egg binding need not involve antibody specific for the sperm receptor, and that antibodies to the zona pellucida block sperm access by steric hinderance. Other known functions of the zona were unaffected. The antibodies were unable to induce the biochemical changes in the zona associated with the postfertilization block to polyspermy and had no detectable effect on preimplantation development.

3/AB/37 (Item 1 from file: 5)
DIALOG(R) File 5:Biosis Previews(R)
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10102180 BIOSIS NO.: 199598557098
Immunocontraception of captive exotic species. I. Przewalski's horses (*Equus przewalskii*) and banteng (*Bos javanicus*).
AUTHOR: Kirkpatrick Jay F(a); Zimmermann Waltraut; Kolter Lydia; Liu I K M; Turner J W Jr
AUTHOR ADDRESS: (a)Sci. Conservation Biol. Program, ZooMontana, P.O. Box 80905, 2100 S. Shiloh Rd., Billings, MT 59**USA
JOURNAL: Zoo Biology 14 (5):p403-416 1995
ISSN: 0733-3188
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A contraceptive vaccine made of porcine zonae pellucidae (PZP) was tested in three Przewalski's mares and five banteng cows. The vaccine antigen consisted of the complete family of glycoproteins of the porcine zona pellucida, including the sperm receptor ZP3. All mares and three of five banteng were inoculated with 2 or 3 i.m. injections of approximately 65 μ -g of antigen given over a 6 week period. Two other banteng received inoculations of only 35 μ -g of antigen on the same schedule. Two of the three mares and three of five banteng cows were pregnant at the time of inoculation. No new pregnancies, as a result of postinoculation breedings, occurred among either the mares 36 months after 65 μ -g antigen inoculations or among the banteng for 24 months after 65 μ -g inoculations. One postinoculation pregnancy resulted among the two banteng receiving only 35 μ -g of antigen. Differences in fertility between treated and control mares and between preinoculation and postinoculation reproductive performance of the banteng were significant ($P < 0.05$). Urinary ovarian steroid metabolites and behavioral observations indicated follicular development and ovulations were occurring among treated mares during the year following PZP inoculations. PZP immunization produced progressively elevated anti-PZP antibodies in both species, which provided contraceptive protection. PZP immunization appears to be an effective form of contraception in both species.

1995

3/AB/38 (Item 2 from file: 5)
DIALOG(R) File 5:Biosis Previews(R)
(c) 2001 BIOSIS. All rts. reserv.

04352385 BIOSIS NO.: 000078081929
MONO CLONAL ANTIBODIES TO THE MAJOR PROTEIN OF THE MURINE ZONA PELLUCIDA
EFFECTS ON FERTILIZATION AND EARLY DEVELOPMENT
AUTHOR: EAST I J; MATTISON D; DEAN J
AUTHOR ADDRESS: LAB. CHEM. BIOL., NIADDK, BETHESDA, MD. 20205.
JOURNAL: DEV BIOL 104 (1). 1984. 49-56. 1984
FULL JOURNAL NAME: Developmental Biology
CODEN: DEBIA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The growing murine oocyte is surrounded by an extracellular zona pellucida consisting of 3 sulfated glycoproteins, ZP-1, ZP-2 and ZP-3. The smallest of these, ZP-3, was reported to be the species-specific sperm receptor. Monoclonal antibodies were recently characterized to 3 different antigenic determinants, 2 found exclusively on ZP-2, and 1 found on both ZP-2 and ZP-3. The in vivo effect of these antibodies on the 3 known functions of the zona pellucida were examined. The most dramatic effect was the prevention of fertilization. After administration, the monoclonal antibodies were located in the ovary on the zona pellucida of growing oocytes. Eggs ovulated subsequently were coated with the monoclonal antibodies and failed to develop into 2 cell embryos after mating. A total of 80 days later, the monoclonal antibodies could no longer be detected on the zona of ovarian oocytes, and this loss coincided with the resumption of fertility. These findings provide molecular evidence for the hypothesis that the immunological block to sperm-egg binding need not involve antibody specific for the sperm receptor, and that antibodies to the zona pellucida block sperm access by steric hinderance. Other known functions of the zona were unaffected. The antibodies were unable to induce the biochemical changes in the zona associated with the postfertilization block to polyspermy and had no detectable effect on preimplantation development.

1984

3/AB/39 (Item 1 from file: 10)
DIALOG(R) File 10:AGRICOLA
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3415329 20436019 Holding Library: AGL
Species-specific binding of sperm proteins to the extracellular matrix (Zona pellucida) of the egg
Hardy, D.M. Garbers, D.L.
Bethesda, Md. : American Society for Biochemistry and Molecular Biology.
The Journal of biological chemistry. July 22, 1994. v. 269 (29) p.
19000-19004.
ISSN: 0021-9258 CODEN: JBCHA3
DNAL CALL NO: 381 J824
Language: English

The zona pellucida is an extracellular matrix surrounding the mammalian egg where species-specific gamete recognition and signaling occur. Pig zona pellucida were isolated in large amounts and used as an affinity matrix for detergent-solubilized, biotinylated membrane proteins of pig spermatozoa. On non-reducing SDS-polyacrylamide gel electrophoresis, specifically bound sperm proteins migrated with Mr 170,000, 150,000, 130,000, 56,000, and

50,000 (p50). Disulfide bond reduction separated each of the Mr 130,000-170,000 proteins into Mr 105,000 (p105) and Mr 45,000 (p45) subunits, indicating that these high Mr proteins are related. Based on two-dimensional electrophoresis, the Mr 56,000 band was composed of three to four proteins that migrated with Mr 56,000-62,000 (p56-62) in the second (reducing) dimension. p50 bound to heterologous zona pellucida (murine, bovine) and to *Xenopus laevis* oocyte envelopes, demonstrating a lack of species specificity to its binding and was identified as proacrosin/acrosin based on amino acid sequences of two tryptic peptides and its interaction with monospecific antibodies to proacrosin. In contrast, p105/p45 and one or more of the p56-62 proteins bound to pig zona pellucida but not to the egg extracellular matrices of the other species; these proteins therefore exhibited the species-specific binding to the zona pellucida expected for molecules involved in specific gamete adhesion. Amino acid sequences of nine tryptic peptides derived from p105/p45 did not match peptide sequences in existing databases, establishing it as a unique protein. These (p105/p45 and at least one p56-62 protein) are the first sperm membrane proteins to be identified that bind in a species-specific manner to the egg extracellular matrix.

3/AB/40 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2001 Inst for Sci Info. All rts. reserv.

03864792 Genuine Article#: QM362 Number of References: 41
Title: ZONA-PELLUCIDA AS A TARGET FOR IMMUNOCONTRACEPTION (Abstract Available)
Author(s): AFZALPURKAR A; KOLLURI SK; KAUL R; BAGAVANT H; GUPTA M; GUPTA SK
Corporate Source: NATL INST IMMUNOL/NEW DELHI 110067//INDIA/; INDIAN VACCINES CORP LTD/NEW DELHI 110014//INDIA/
Journal: CURRENT SCIENCE, 1995, V68, N4 (FEB 25), P440-445
ISSN: 0011-3891
Language: ENGLISH Document Type: ARTICLE
Abstract: The zona pellucida (ZP) has generated considerable interest as a target for the immunocontraceptive vaccine, blocking pregnancy at pre-fertilization stage. Antibodies against porcine ZP3 have been shown to inhibit sperm-egg interaction. The immunological cross-reactivity among the various species of ZP glycoproteins, has led to the possibility of heterologous immunization. Recent studies in nonprimates using purified ZP components reversible infertility without side effects. More recently, peptide immunogens based on the ZP sequence, have become candidate contraceptive vaccines with the demonstration that the deglycosylated ZP components can block fertility with reduced ovarian dysfunction.

3/AB/41 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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03098321 Genuine Article#: ND771 Number of References: 38
Title: CHANGES IN MORPHOLOGY, SPERM PENETRATION AND FERTILIZATION OF OVULATED HAMSTER EGGS INDUCED BY OVIDUCTAL EXPOSURE (Abstract Available)
Author(s): BOATMAN DE; FELSON SE; KIMURA J
Corporate Source: UNIV WISCONSIN, DEPT ANIM HLTH & BIOMED SCI, 1655 LINDEN

DR/MADISON//WI/53706; NIHON UNIV, COLL AGR & VET MED, DEPT VET
ANAT/FUJISAWA/KANAGAWA/JAPAN/

Journal: HUMAN REPRODUCTION, 1994, V9, N3 (MAR), P519-526

ISSN: 0268-1161

Language: ENGLISH Document Type: ARTICLE

Abstract: In the human, mature eggs at the pre-ovulatory follicular stage placed into the oviduct via gamete intra-Fallopian transfer (GIFT) establish more implantations and pregnancies than do those for standard in-vitro fertilization and embryo Transfer (ICF). Previous studies in the hamster have shown that mature follicular eggs are less readily penetrated by spermatozoa than oviductal eggs. This study examines whether ovulation or pre-fertilization exposure to the oviduct per se affects sperm penetration and fertilization of mature ova. Three types of eggs were used: pre-ovulatory, follicular [12 h post-human chorionic gonadotrophin (HCG), 1-1.5 h pre-ovulation], and ovulated (bursal and oviductal, both 15 +/- 0.5 h post-HCG). Bursal eggs were obtained by ligating the infundibulum on one or both sides of the tract. The morphological changes in eggs due to oviductal exposure were quantified using computerized image analysis. Cumulus-free follicular and bursal eggs were significantly less penetrated than oviductal eggs 1 h post-insemination (36, 39 and 62%, respectively). Cumulus-intact oviductal compared to bursal eggs, paired within females, were fertilized at a significantly higher rate (4 h post-insemination; 89 and 58%, respectively). Fresh oviductal and bursal eggs had equivalent cell diameters (79 μ m) and zona thickness (15-15.8 μ m), but oviductal compared with bursal eggs had larger zonae (119 and 116 μ m, respectively) and perivitelline volumes (107 and 47 μ l). Oviductal, but not bursal, zonae had the oviductal glycoprotein, oviductin, bound to them. We conclude that prefertilization oviductal exposure and not ovulation or time post-HCG alters the morphology and fertilizability of eggs.

3/AB/42 (Item 1 from file: 50)

DIALOG(R) File 50:CAB Abstracts

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02452038 CAB Accession Number: 910190706

Analysis of the antigenicity of hamster zona pellucida with a monoclonal antibody.

Oikawa, T.

Developmental and Reproductive Biology Center and Bio Science Laboratory, 5-34-5, Shironishi-machi, Yamagata 990, Japan.

Conference Title: Reproductive immunology 1989. Proceedings of the 4th International Congress on Reproductive Immunology, Kiel, Germany, 26-29 July 1988

p.109-116

Publication Year: 1990

Editors: Mettler, L.; Billington, W. D.

Publisher: Elsevier Science Publishers -- Amsterdam, Netherlands

ISBN: 0-444-81153-2

Language: English

Document Type: Conference paper

Several studies carried out on hamsters in the author's laboratory are summarised. Electrophoretic studies showed that the zona pellucida derived from ovarian eggs differed from that of oviductal eggs. Fluorescence studies on ovarian eggs, oviductal eggs and ovarian eggs

treated with oviduct extract suggested that a factor present in the oviduct, named egg surface modifying glycoprotein (ESMGp), was responsible for changing the biochemical properties of zona pellucida of eggs after ovulation. Ultrastructural studies showed that ESGMp was synthesised by the epithelial cells of the inner wall of the oviduct, and was then secreted into the oviduct. Using polyclonal antibodies against ESGMp, raised in rabbits, it was shown that the factor on the oviductal zona pellucida (Zp-0) was the same as ESGMp. In vitro fertilization of hamster ovarian eggs, oviductal eggs and ovarian eggs treated with Zp-0, using hamster spermatozoa, resulted in fertilization rates of 100, 30 and 100% resp. indicating that Zp-0 promotes sperm binding. 9 ref.

3/AB/43 (Item 2 from file: 50)
DIALOG(R) File 50:CAB Abstracts
(c) 2001 CAB International. All rts. reserv.

01709560 CAB Accession Number: 860195353

Biosynthesis of the mouse zona pellucida and the effect of anti-zona monoclonal antibodies on fertilization and early development.

Dean, J.; East, I. J.; Shimizu, S.

Laboratory of Cellular and Developmental Biology, NIADDK, NIH, Bethesda, Maryland 20205, USA.

Theriogenology vol. 25 (1): p.107-116

Publication Year: 1986

ISSN: 0093-691X --

Language: English

Document Type: Conference paper; Journal article

The mouse zona pellucida consists of 3 sulphated glycoproteins (ZP-1, ZP-2 and ZP -3) which are synthesised during oogenesis and are secreted to form an extracellular matrix which mediates sperm-egg interactions and appears to protect the growing embryo as it passes down the oviduct. Parenterally administered anti-ZP-2 and anti- ZP - 3 monoclonal antibodies were found only in the ovary where they had attached to the zona pellucida surrounding the growing oocytes. When ovulated, these ova could bind spermatozoa, but the presence of the antibody effectively prevented sperm penetration of the zona pellucida and, thus, inhibited fertilization. This effect continued until all the antibody-bound oocytes had ovulated (approx equal to 60 days), at which time the treated animals recovered their fertility. 18 ref.

3/AB/44 (Item 1 from file: 77)
DIALOG(R) File 77:Conference Papers Index
(c) 2001 Cambridge Sci Abs. All rts. reserv.

4154219

Supplier Accession Number: 95-04077

V23N04

Association of human oviductal glycoprotein with ovarian oocytes influences sperm binding to the zona pellucida

O'Day-Bowman, M.B.; Mavrogianis, P.A.; Reuter, L.M.; Johnson, D.; Fazleabas, A.T.; Verhage, H.G.

Dept. Obstet. and Gynecol., Univ. Illinois at Chicago, Chicago, IL, USA

42nd Annual Meeting of the Society for Gynecological Investigation

9515006 Chicago, IL 15-18 Mar 1995

Society for Gynecologic Investigation

Elsevier Science Inc., 655 Avenue of the Americas, New York, NY 10010,
Abstracts available. Paper No. 06

3/AB/45 (Item 1 from file: 144)
DIALOG(R) File 144:Pascal
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12853580 PASCAL No.: 97-0074399
Antibody responses and infertility in mice following oral immunization
with attenuated Salmonella typhimurium expressing recombinant murine ZP3
SUP 1

ZHANG X; LOU Y H; KOOPMAN M; DOGGETT T; TUNG K S K; CURTISS R III
Department of Biology, Washington University, St. Louis, Missouri 63130,
United States; Department of Pathology, University of Virginia Health
Sciences Center, Charlottesville, Virginia 22909, United States
Journal: Biology of reproduction, 1997, 56 (1) 33-41
Language: English

Ovarian ZP3, the primary sperm receptor, is a major glycoprotein
of mouse zona pellucida (ZP). Because antibodies raised against ZP3
block sperm-egg interaction, ZP3 has been considered a candidate
immunogen in the development of a contraceptive vaccine. This study
explored the possibility of using an attenuated Salmonella typhimurium
vaccine strain expressing recombinant ZP3 to elicit an antibody response
and infertility in mice. A cDNA sequence generated by the polymerase chain
reaction encoding 342 amino acid residues (23-364) of the mouse (m)ZP3
was cloned into an Asd SUP + vector. An avirulent Salmonella vaccine strain
stably expressed the ZP3 polypeptide and colonized the internal organs of
mice after oral inoculation. Oral immunization of female BALB/c mice with
the recombinant Salmonella vaccine strain expressing mZP3 induced
significant levels of anti-native ZP IgG antibodies in serum and IgA
antibodies in vaginal secretions. The IgG antibodies thus induced also
bound to ZP in vivo. When mated with males, 3 of 6 females immunized with
the recombinant Salmonella were infertile. In contrast, none of the mice
that received Salmonella containing the vector plasmid produced antibodies
to ZP and all were fertile. No ovarian inflammation was observed in the
immunized mice at autopsy. The results suggest a potential oral
contraceptive vaccine to control populations of rodent vectors of disease
and to induce reversible infertility in humans.

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3/AB/46 (Item 1 from file: 149)
DIALOG(R) File 149:TGG Health&Wellness DB(SM)
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01119115 SUPPLIER NUMBER: 04674251 (USE FORMAT 7 OR 9 FOR FULL TEXT)
The biology and chemistry of fertilization. (mice prototype for mammals;
research on mechanisms)
Wassarman, Paul M.
Science, v235, p553(8)
Jan 30,
1987

PUBLICATION FORMAT: Magazine/Journal ISSN: 0036-8075 LANGUAGE: English
RECORD TYPE: Fulltext TARGET AUDIENCE: Academic
WORD COUNT: 4440 LINE COUNT: 00449

